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TRANSLOCATION OF CARBOHYDRATES IN THE CUTHBERT RASPBERRY[1]

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(WITH FOUR FIGURES)

Introduction

Many investigations have been conducted on the raspberry plant, especially in regard to the economically important aspects such as resistance to freezing, pruning, fertilizing, etc., but there has been little study relative to the forms and movements of the chief organic substances. DU SABLON (15) included the raspberry in his study of the reserve materials of trees. BRIERLEY (2, 3) studied senescence in the red raspberry cane, emphasizing cambial decline and phloem disintegration, and the responses to pruning. BRIERLEY and LANDON (4) presented evidence on the downward movement of carbohydrates in the fruiting canes. BENNETT (1) demonstrated that the movement of the leaf-curl virus of raspberries takes place in the phloem and in the direction of the major transport of food. Reviews of the literature in translocation have been published by CLEMENTS (8), CURTIS (14), and more recently by MASON and PHILLIS (22) and CRAFTS (13).

Material and methods

The Cuthbert raspberry is a hybrid derived from *Rubus idaeus* L. and *R. strigosus* Michx., the latter considered by HEDRICK (17) as a

[1] Contribution no. 57 from the Botany Department of the State College of Washington.

subspecies of *R. idaeus*. It produces tall erect canes which attain their full height and most of their diameter growth within the first growing season. Fruiting occurs in the second season on laterals developed from over-wintered buds. At the height of the harvest season disintegration of the phloem occurs at the base and in the apical region of the cane. BRIERLEY (2) thinks the former is associated with the senile condition of the cambium, and the latter caused by a disturbance in water conduction.

Because the various canes are relatively uniform in size, length, and diameter, the Cuthbert raspberry was chosen for experimental material. It was at first intended to include second year (fruiting) canes as well as first year ones, thus making a complete study up to and beyond fruiting; but during the winter previous to the beginning of this study all canes were winter killed, and none which could be used during the following growing season could be found in the locality.

The experimental plants were mosaic-free stock grown by the Department of Horticulture at the State College of Washington.[2] They were grown on a uniform Palouse silt loam soil with ample moisture for growth, and on a southwest slope. The rainfall of this region comes mostly during the winter and spring; after May gradual drying of the soil occurs up to the end of September, when there are occasional showers.

In this study double ringing was employed. By ringing at a point 15 cm. and again at a point 30 cm. above the ground, a segment of bark 15 cm. in length was isolated. The strips of bark removed were less than 1 cm. wide, completely girdling the stem. The lower ring prevents upward movement in the bark of certain substances from the roots and other normal canes growing therefrom, and the upper ring prevents downward movement of materials mobile in the bark into the isolated segment below.

On May 15, 1936, when the new shoots were about 60 cm. high, some were ringed, some tagged for controls, and others were taken into the laboratory and prepared for subsequent analysis. The plants harvested at the time of ringing on May 15 served as checks

[2] Thanks are due to the Department of Horticulture, State College of Washington, for the raspberry plants used throughout this investigation.

for the June series. Ringed canes and the controls tagged on May 15 were harvested on June 23, and another set of canes were ringed and others tagged for additional controls on this date. These were harvested on July 16 and constitute the July series. On July 16 canes were ringed, and others tagged for controls; these were harvested on August 9, as the August series. Control plants for any series (except May) were those which were tagged but not ringed in the immediately preceding month. Twenty-four canes were used in each treatment, twelve for nitrogen and twelve for carbohydrate analyses.

At each collection the canes were severed at the ground level. Each cane was cut into 15 cm. segments, measuring from the base, so that the sample contained the equivalent segment of twelve canes. The leaves of each segment were cut off at the junction of petiole and stem and were analyzed in aggregates of twelve equivalent segments. The stem samples were analyzed separately. The top segment (or portion thereof) and its young leaves were analyzed as one sample. For these various samples the following symbols have been adopted: (1) Each 15 cm. segment of stem, numbering from the base to the top, is designated as 1st, 2st, 3st, etc. Thus in the double ringed canes, segment 1st is that below the lower ring; segment 2st is that between the rings; and segment 3st is that above the upper ring. (2) The several leaves diverging from each segment are symbolized as 3L, 4L, 5L, and 6L, these being from segments 3st, 4st, 5st, and 6st respectively. The leaves usually drop from the first 30 cm. of the raspberry stem, so that in these experiments the leaves were cut from the first two segments at each treatment. (3) The uppermost segment (or portion thereof) with leaves too small to constitute a separate sample, is designated as tops.

The segmentation was done as rapidly as possible, and all samples were immediately weighed and autoclaved at 15 lb. pressure for 15 minutes. After drying in a ventilated oven heated to 60° C., the material for carbohydrate determinations was ground to 40-mesh fineness in a power mill.

Moisture.—The moisture content of the carbohydrate samples was determined by drying them for a week in an oven at 60° C. and then weighing.

CARBOHYDRATES.—The procedure was essentially that described by CLEMENTS (6), with the exception that the term "acid hydrolyzable carbohydrates" has been substituted for "hemicellulose." This group was taken out in two fractions (11), one after three hours of hydrolysis on the steam bath and the second after twelve hours. The first group, designated as "acid hydrolyzable A," is presumably made up of polysaccharides of high molecular weight which are held more or less loosely to the cell wall or within the protoplast. The second group, "acid hydrolyzable B," is made up of polysaccharides which are more stable and which are possibly part of the secondary thickening of the cell wall.

TABLE 1

DISTRIBUTION OF CARBOHYDRATES IN NORMAL PLANTS
MAY 15 (BEGINNING CONTROLS)

CANE SEGMENT	TOTAL CARBOHYDRATE		REDUCING SUGARS		SUCROSE		STARCH		TOTAL ACID HYDROLYZABLE	
	GM.	RESIDUAL DRY WEIGHT (%)	GM.	RESIDUAL DRY WEIGHT (%)	GM.	RESIDUAL DRY WEIGHT (%)	GM.	RESIDUAL DRY WEIGHT (%)	GM.	RESIDUAL DRY WEIGHT (%)
1st.....	5.80	27.70	1.40	06.0	0.06	0.24	0.18	0.77	4.16	17.70
2st.....	4.98	31.13	1.67	10.4	0.07	0.46	0.23	1.44	2.91	18.18
3st.....	2.72	33.18	1.20	14.7	0.07	0.83	0.18	2.20	1.27	15.49
Tops.....	2.53	33.72	1.04	13.9	0.05	0.71	0.18	2.40	1.26	16.80
3L.....	12.20	36.40	4.94	14.1	0.05	0.15	0.07	0.21	5.71	17.05

EXPRESSION OF RESULTS.—The basis upon which to calculate and report analytical results is a prevalent controversy. In the light of considerations to be reported elsewhere (16), the following procedure is used in expressing results: (1) When it is especially desirable, absolute changes in a segment are expressed as the weight difference in grams per sample (equivalent segments of twelve canes = a sample). (2) Percentage concentrations are used in most cases, such as those dealing with gradients. The substances of the control plants are expressed as percentages of their residual dry weight. The substances of the treated plants are expressed as percentages of the residual dry weight of the corresponding controls.

Investigation

Seasonal distribution of carbohydrates in normal plants

Total carbohydrates.—The percentage of total carbohydrates, which includes the material hydrolyzable in 2.5 per cent acid in fifteen hours, is fairly high in the stem and leaves of the plants harvested in May (table 1). It drops during June, the period of most

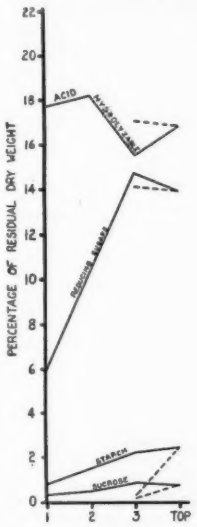

Fig. 1

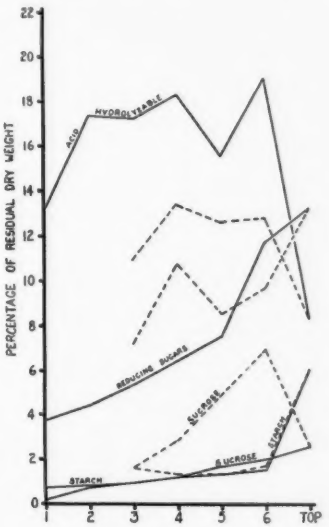

Fig. 2

Figs. 1, 2.—Fig. 1 (left), distribution of carbohydrates in normal cane segments, May 15. Dotted lines represent leaf samples corresponding to stem segments. Cane segments, numbering from base of stem to top, are indicated along abscissa. Fig. 2 (right), distribution of carbohydrates in normal cane segments, June 23.

rapid growth, and attains a maximum in August. The drop during the period of rapid growth corresponds to that observed by Du Sablon (15) in the raspberry. He attributed the early spring high to a migration of carbohydrates from the roots into the stems. He obtained a maximum of total carbohydrates, as percentages of the dry weight, in October. The concentrations of total carbohydrates are slightly lower in the lower segments than in the upper in the May

and June collections. This ascending order practically disappears during July and August. All fractions of the total carbohydrates for the normal plants are plotted in figures 1 to 4.

ACID HYDROLYZABLE CARBOHYDRATES.—This group of substances, the largest fraction of the total carbohydrates, made up of heterogeneous cellulosic compounds—polysaccharides of high molecular

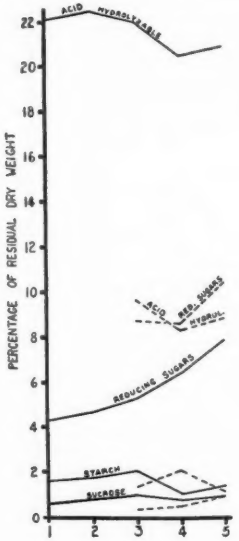

FIG 3

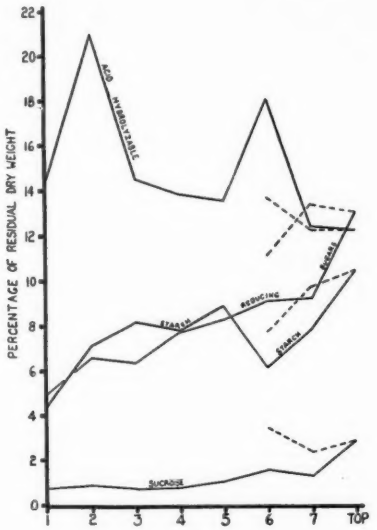

FIG. 4

FIGS. 3, 4.—Fig. 3 (left), distribution of carbohydrates in normal cane segments, July 16. Fig. 4 (right), same, August 9.

weight and their derivatives—have varying degrees of stability upon hydrolysis. For this reason, hydrolysis for three hours and then for an additional twelve hours yielded very erratic results. For example, the concentration of the former or A fraction varied from 1 per cent in one segment to 10 per cent in the neighboring segment, whereas the second or B fraction would be the reverse. The total of the two fractions in any one segment, however, was comparable with that of the other segments. The idiosyncrasies of the less stable "hemicelluloses" disappeared upon continued hydrolysis, although some in-

consistency still remained. For this reason the individual A and B fractions of the acid hydrolyzable substances were not graphed nor tabulated. Separate hydrolysis, however, has the advantage of largely preventing the destruction of that portion of the hydrolysate which is produced early in the boiling process.

With the exception of the May series, the tops are lowest in concentration of these substances. The second segment is always considerably higher in concentration than the first, and with the exception of the June series the concentration in the second segment is the highest of all parts of the plant. This may be owing to its location just below the foliage region, for, as BRIERLEY (2) has shown for fruiting canes, cambial activity is greatest in the region below a vigorous lateral or foliage, and is usually absent in the base of the cane. In 90 per cent of the canes measured by BRIERLEY (2) he found no measurable increase in diameter at the base of old canes. There may be some relationship between the erratic activity of the raspberry cambium in various regions of the stem and the erratic results obtained for the acid hydrolyzable material along the stem, but the results are probably caused by the variable responses of the substances to hydrolysis.

In all but the May series the amount of acid hydrolyzable material is lower in the leaves than in the stem segments. The increase of the acid hydrolyzable fraction in the tops of the May series is accompanied by a drop in the reducing sugars. The graph of this drop (fig. 1) from 3st to tops, and the leaves of 3st to tops, is nearly a mirror image of the graph of the acid hydrolyzable substances in the same region. The amounts of reducing sugars in this series are higher than in any other collection, but starch and sucrose are lower. The highest concentration of sugars is nearly 700 per cent higher than that of starch. There seems to be a close relationship in the May series between the high concentrations of reducing sugars and the acid hydrolyzable carbohydrates. This indicates that not only are the "hemicelluloses" a form of food reserve, but they may be an even more immediate storage form than starch. Thus it is concluded with CLEMENTS (6) that, "the vacuolar sugars may be so concentrated as to favor the formation of some reserve which is entirely removed from the solution," and that, "under favorable conditions

for rapid carbohydrate formation they [the hemicelluloses] seem to act as temporary reserves in the leaves."

REDUCING SUGARS.—The highest concentrations of reducing sugars are in the May series, drop rapidly in the June series, from whence they increase only slightly in July and August. The basal segment is always lowest in reducing sugars, with concentrations increasing toward the top. The leaves diverging from the subterminal segment have less sugars than that segment, except in the August series. Lower leaves are always higher in concentration of sugars than their corresponding segment. The large amount of reducing sugars in all parts of the plant seems to indicate that it is an important temporary storage form.

STARCH.—Starch is never abundant, and does not rise above 6 per cent except in the August series, where it essentially parallels the reducing sugar curve. Apparently it is not an important reserve in the first year canes of raspberry. The starch content of leaves is lower than that of the corresponding stem segments in May, is equivalent in June, slightly above in July, and definitely higher in August. The gradient is not steep and the basal segment is lowest in starch content.

SUCROSE.—Sucrose is present at all times in all parts of the plant, but its concentration is low, rising above 2 per cent only in June and in August. It becomes highly concentrated in the leaves of the June series, reaching a maximum in the uppermost and grading downward to the lowermost leaves.

The very low concentration of this sugar indicates that it is not an important one, either for direct use as an energy source or for temporary storage.[3] Its presence throughout the plant suggests that it has a definite role in metabolism, probably of translocation. That it does not result as a condensation product when reducing sugars attain a certain maximum concentration, such as CLEMENTS (**6**) found in his work on the sunflower, soy bean, and potato, is indicated by an entire lack of relationship between the varying concentrations of reducing sugars and the little-fluctuating amounts of sucrose. There seems to be no relationship with moisture content, as LEONARD (**18**)

[3] Microchemical data to be presented in a later paper indicate that sucrose is limited in its distribution, essentially to phloem, making it inconceivable that sucrose functions as an important source of energy.

found in the sunflower. The very small amounts of sucrose in the stem indicate that, if it is a translocatory form, it is confined to a very small part of the total mass of stem, presumably the phloem. The actual concentrations in this tissue are thus greatly reduced by the total mass of the stem. The slight gradient which appears in figures 1 to 4 is insignificant and is probably nonexistent. It is the result of dividing the grams of sucrose by residual dry weights which are somewhat greater in the lower than in the upper portions of the plant.

Effect of ringing

The effects of double ringing on the sugars in the treated plants are compared with the untreated plants in tables 2 to 4. The usual accumulation of carbohydrates is found in the parts above the rings owing to the prevention of movements into regions below the ring. According to Mason and Maskell (**20**), ringing is noticeably effective in the interruption of the downward movement of carbohydrates within two hours. In all series there is a great increase of total carbohydrates above the rings over the normal concentrations. Accompanying this increase is an even greater decrease of total carbohydrates between and below the rings.

Increases or decreases in the ringed canes, expressed in whole numbers as percentages of the normal concentrations, are given for the segments below the rings (1st), between the rings (2st), and just above the rings (3st) in table 5. It is evident that all carbohydrate substances, except starch in the June series, make large percentage increases in the segment above the upper ring. The largest increase was in the amount of sucrose. The percentage increase of reducing sugars was only half that made by sucrose.

All fractions decrease greatly between the rings. Sucrose again was affected most, there being a 75 per cent reduction in June, and a 100 per cent reduction in July and August. The reducing sugars showed not so great an apparent decrease, but much of that present at the time of analysis was undoubtedly derived from starch and acid hydrolyzable carbohydrates, as well as from the residual polysaccharides (table 7). The data in tables 5 and 7 indicate that carbohydrates other than reducing sugars were 43 to 100 per cent lower than normal.

TABLE 2

SEASONAL CHANGES IN TOTAL CARBOHYDRATES AND REDUCING SUGARS IN NORMAL AND RINGED PLANTS

CANE SEGMENT	JUNE 23				JULY 16				AUGUST 9			
	CONTROLS		RINGED		CONTROLS		RINGED		CONTROLS		RINGED	
	GM.	RESIDUAL DRY WEIGHT (%)	GM.	CONTROL RESIDUAL DRY WEIGHT (%)	GM.	RESIDUAL DRY WEIGHT (%)	GM.	CONTROL RESIDUAL DRY WEIGHT (%)	GM.	RESIDUAL DRY WEIGHT (%)	GM.	CONTROL RESIDUAL DRY WEIGHT (%)
	TOTAL CARBOHYDRATES											
1st	4.00	17.75	1.67	7.44	6.60	28.41	3.82	16.47	8.20	35.68	5.10	22.15
2st	3.70	22.98	1.13	6.99	5.10	29.48	2.81	16.23	6.40	35.55	2.28	12.67
3st	3.30	23.73	5.12	36.85	4.40	30.55	5.54	38.40	4.80	29.45	7.73	47.40
4st	3.10	28.43	4.27	39.25	2.80	28.90	4.52	46.70	3.90	30.00	7.02	54.00
5st	2.10	26.25	3.03	37.85	2.50	31.28	4.14	51.75	3.10	31.60	4.33	44.30
6st	1.40	34.15	1.93	47.10	....	.....	....	.....	2.70	34.60	4.01	51.40
7st	....	.....	....	.....	....	.....	....	.....	1.90	30.15	2.79	44.30
Top	3.80	29.45	3.55	27.50	....	.....	....	.....	5.90	38.80	6.95	45.70
7L	....	.....	....	.....	....	.....	....	.....	6.10	37.45	13.85	85.00
6L	5.20	30.75	5.45	32.20	....	.....	....	.....	6.30	36.22	15.44	88.80
5L	5.60	27.45	5.87	28.80	5.80	29.00	6.10	30.50	....	.....	....	.....
4L	5.40	28.28	6.10	31.95	4.20	23.60	5.70	32.20	....	.....	....	.....
3L	3.50	21.07	5.17	31.15	4.60	22.45	....	.....	....	.....	....	.....
	REDUCING SUGARS											
1st	0.85	3.78	0.37	1.64	0.99	4.27	1.28	5.52	1.14	4.96	0.93	4.04
2st	0.71	4.40	0.23	1.45	0.82	4.64	0.61	3.53	1.18	6.55	0.49	2.72
3st	0.74	5.35	1.52	10.90	0.76	5.28	1.24	8.61	1.03	6.30	2.75	16.87
4st	0.70	6.43	1.24	11.40	0.62	6.39	0.95	9.80	1.01	7.77	2.06	15.85
5st	0.60	7.50	1.14	14.20	0.63	7.88	1.05	13.14	0.81	8.27	1.50	15.32
6st	0.47	11.75	0.76	18.45	....	.....	....	.....	0.71	9.10	1.09	14.00
7st	....	.....	....	.....	....	.....	....	.....	0.58	9.20	1.06	16.82
Top	1.70	13.19	1.64	12.70	....	.....	....	.....	1.98	13.02	3.02	19.88
7L	....	.....	....	.....	....	.....	....	.....	2.17	13.30	6.00	36.80
6L	1.63	9.64	2.09	12.35	....	.....	....	.....	1.94	11.15	6.29	36.15
5L	1.75	8.58	2.24	10.95	2.08	10.40	2.25	11.30	....	.....	....	.....
4L	2.06	10.78	2.49	13.04	1.52	8.54	1.84	10.33	....	.....	....	.....
3L	1.18	7.11	2.34	14.53	1.80	8.77	....	.....	....	.....	....	.....

TABLE 3

SEASONAL CHANGES IN SUCROSE AND STARCH IN NORMAL AND RINGED PLANTS

Cane segment	June 23				July 16				August 9			
	Controls		Ringed		Controls		Ringed		Controls		Ringed	
	Gm.	Residual dry weight (%)	Gm.	Control residual dry weight (%)	Gm.	Residual dry weight (%)	Gm.	Control residual dry weight (%)	Gm.	Residual dry weight (%)	Gm.	Control residual dry weight (%)
	Sucrose											
1st	0.03	0.133	0.092	0.408	0.13	0.561	0.354	1.525	0.16	0.695	0.378	1.641
2st	0.11	0.684	0.027	0.167	0.13	0.752	0.000	0.000	0.15	0.834	0.000	0.000
3st	0.12	0.863	0.454	3.270	0.14	0.972	0.312	2.165	0.11	0.675	0.422	2.530
4st	0.13	1.192	0.326	2.990	0.07	0.721	0.311	3.200	0.10	0.770	0.883	6.790
5st	0.13	1.625	0.262	3.270	0.07	0.875	0.291	3.540	0.10	1.020	0.336	3.430
6st	0.08	1.950	0.213	5.180	....	.....	.....	.....	0.12	1.540	0.683	8.750
7st	....	.....	.....	.....	....	.....	.....	.....	0.08	1.260	0.149	2.360
Top	0.32	2.480	0.694	5.380	....	.....	.....	.....	0.43	2.830	1.826	12.000
7L	....	.....	.....	.....	....	.....	.....	.....	0.39	2.390	1.755	10.875
6L	1.17	6.930	1.119	6.620	....	.....	.....	.....	0.60	3.430	2.810	16.125
5L	1.00	4.910	1.850	9.070	1.75	0.875	0.693	3.465	....	.....	.....	.....
4L	0.54	2.830	1.498	7.850	0.81	0.456	0.713	4.000	....	.....	.....	.....
3L	0.27	1.623	0.891	5.375	0.59	0.288	.....	.....	....	.....	.....	.....
	Starch											
1st	0.15	0.667	0.000	0.000	0.37	1.595	0.112	0.482	1.00	4.350	1.111	4.840
2st	0.12	0.745	0.025	0.155	0.30	1.735	0.000	0.000	1.28	7.120	0.160	0.890
3st	0.12	0.854	0.064	0.460	0.29	2.015	0.645	4.482	1.33	8.160	1.870	11.475
4st	0.13	1.191	0.056	0.511	0.10	1.048	0.608	6.270	1.01	7.770	1.825	14.050
5st	0.10	1.250	0.062	0.769	0.11	1.376	0.633	7.910	0.86	8.780	1.062	10.850
6st	0.06	1.462	0.028	0.685	....	.....	.....	.....	0.48	6.160	1.044	13.400
7st	....	.....	.....	.....	....	.....	.....	.....	0.50	7.940	0.853	13.550
Top	0.78	6.050	0.166	1.289	....	.....	.....	.....	1.59	10.460	0.748	4.360
7L	....	.....	.....	.....	....	.....	.....	.....	1.59	9.750	4.360	26.740
6L	0.28	1.657	0.132	0.778	....	.....	.....	.....	1.34	7.710	2.355	13.520
5L	0.27	1.323	0.119	0.582	0.22	1.100	1.300	6.500	....	.....	.....	.....
4L	0.25	1.310	0.105	0.549	0.36	2.020	0.989	5.560	....	.....	.....	.....
3L	0.26	1.568	0.044	0.265	0.28	1.366	.....	.....	....	.....	.....	.....

All fractions except sucrose and the reducing sugars in the July series showed a decrease in the basal segment. In all cases the decrease was less in July and August, owing to the duration of the treatment for only three weeks instead of five and a half weeks as in the June series.

TABLE 4

SEASONAL CHANGES IN ACID HYDROLYZABLE SUBSTANCES IN NORMAL AND RINGED PLANTS

Cane segment	June 23				July 16				August 9			
	Controls		Ringed		Controls		Ringed		Controls		Ringed	
	Gm.	Residual dry weight (%)	Gm.	Control residual dry weight (%)	Gm.	Residual dry weight (%)	Gm.	Control residual dry weight (%)	Gm.	Residual dry weight (%)	Gm.	Control residual dry weight (%)
1st	2.97	13.20	1.214	5.355	5.14	22.15	2.077	8.953	3.32	14.43	2.605	11.325
2st	2.78	17.28	0.840	5.212	3.89	22.48	2.204	12.740	3.79	21.05	1.634	9.070
3st	2.39	17.19	3.089	22.230	3.18	22.08	3.343	23.300	2.36	14.47	2.683	16.424
4st	2.00	18.34	2.152	19.750	1.99	20.52	2.640	27.215	1.80	13.85	2.262	17.390
5st	1.24	15.50	1.573	19.660	1.68	21.00	2.177	27.250	1.33	13.57	1.425	14.540
6st	0.78	19.01	0.945	23.020	....	.....	.....	.....	1.41	18.10	1.206	15.449
7st	....	.....	.....	.....	....	.....	.....	.....	0.78	12.39	0.725	11.500
Top	1.07	8.29	1.047	8.114	....	.....	.....	.....	1.86	12.24	1.351	8.888
7L	....	.....	.....	.....	....	.....	.....	.....	2.00	12.25	1.714	10.501
6L	2.15	12.73	2.093	12.385	....	.....	.....	.....	2.41	13.86	3.979	22.880
5L	2.57	12.61	1.693	8.308	1.78	8.90	1.830	9.159	....	.....	.....	.....
4L	2.55	13.35	1.995	10.437	1.48	8.32	2.152	12.083	....	.....	.....	.....
3L	1.82	10.96	1.894	11.410	1.98	9.67	.....	.....	....	.....	.....	.....

Sucrose made remarkable gains in the basal segment, in some instances equaling and even surpassing the increase in the segment above the rings. The large increases above the ring, 100 per cent greater than the increases of reducing sugars, indicate very strongly that sucrose serves only as a translocatory form moving downward in the stem. However, increases of the same magnitude below the rings are equally strong evidence that sucrose is the main translocatory form moving upward from the roots into the stem. Thus ringing brings about a bidirectional movement in the canes, which is con-

TABLE 5

DECREASES (−) OR INCREASES (+) AS PERCENTAGE OF NORMAL IN FIRST THREE SEGMENTS OF RINGED STEMS

FRACTION	CANE SEGMENT	JUNE 23 (%)	JULY 16 (%)	AUGUST 9 (%)
Total carbohydrates	1st	− 58	− 42	− 38
	2st	− 70	− 45	− 81
	3st	+ 57	+ 25	+ 61
Total acid hydrolyzable	1st	− 59	− 59	− 21
	2st	− 70	− 43	− 57
	3st	+ 29	+ 5	+ 13
Reducing sugars	1st	− 53	+ 28	− 25
	2st	− 66	− 24	− 59
	3st	+102	+ 62	+116
Sucrose	1st	+211	+173	+134
	2st	− 75	−100	−100
	3st	+283	+124	+272
Starch	1st	−100	− 70	+ 11
	2st	− 79	−100	− 86
	3st	− 46	+122	+ 41

TABLE 6

GRADIENTS IN REDUCING SUGARS AND SUCROSE IN NORMAL AND RINGED CANES

	JUNE 23					AUGUST 9		
	TOTAL (GM.)	GM./CM.	6L TO 6ST (GM.)	5L TO 5ST (GM.)	6L TO 3L (GM.)	TOTAL (GM.)	GM./CM.	7L TO 7ST (GM.)
	1ST TO TOPS					1ST TO TOPS		
Normal								
Reducing sugars	+9.40	+0.100	−2.20	+1.1	+2.5	+ 8.00	+0.07	+ 4.1
Sucrose	+1.62	+0.018	+4.98	+3.3	+4.3	+ 2.14	+0.02	+ 1.1
	3ST TO TOPS					3ST TO TOPS		
Ringed								
Reducing sugars	+1.8	+0.030	−6.1	−3.0	−2.1	+ 3.0	+0.04	+20.0
Sucrose	+2.1	+0.035	+1.4	+5.8	+1.2	+10.5	+0.14	+14.5

TABLE 7

CHANGES IN RESIDUAL DRY WEIGHT IN FIRST FOUR SEGMENTS OF NORMAL AND RINGED CANES OF JUNE AND AUGUST COLLECTIONS

Cane segment	May 15		June 23						July 16		August 9					
	Normal		Normal		Ringed				Normal		Normal		Ringed			
	Residual dry weight (gm.)	Total carbohydrates (gm.)	Residual dry weight (gm.)	Total carbohydrates (gm.)	Residual dry weight (gm.)	Total carbohydrates (gm.)	Residual dry weight*	Total carbohydrates*	Residual dry weight (gm.)	Total carbohydrates (gm.)	Residual dry weight (gm.)	Total carbohydrates (gm.)	Residual dry weight (gm.)	Total carbohydrates (gm.)	Residual dry weight*	Total carbohydrates*
1st	23.5	5.8	22.5	4.0	8.3	1.7	−63.0	−57.5	23.2	6.6	23.0	8.2	19.4	5.1	−15.6	−37.8
2st	16.0	5.0	16.1	3.7	8.9	1.1	−44.7	−70.4	17.3	5.1	18.0	6.4	13.4	2.3	−25.6	−64.0
3st	8.2	2.7	13.9	3.3	16.2	5.1	+23.7	+54.5	14.4	4.4	16.3	4.8	20.2	7.7	+23.9	+60.4
4st	.....	.....	10.9	3.1	11.5	4.3	+ 5.5	+38.8	9.7	2.8	13.0	3.9	16.8	7.0	+22.6	+79.5

* Increase (+) or decrease (−) as percentage of normal.

firmatory of evidence based on virus activity by BENNETT (1). In addition to the accumulation of sucrose above the rings, an accumulation of reducing sugars also occurs, indicating that some of the latter move downward in the bark.

CARBOHYDRATE GRADIENTS

All carbohydrate gradients in the stem are positive; that is, they descend from a high concentration in the top to a low concentration in the base.[4]

The gradients are positive for insoluble as well as for soluble carbohydrates, and therefore do not give evidence of the direction or the form of movement. Girdling may increase or decrease the gradients but they always remain positive.

As was found by MASON and MASKELL (20, 21), sugars move downward with a marked positive gradient (table 6). Unlike their results, however, is the fact that ringing does not cause the gradient to disappear (table 6). It reduces the positive gradient of reducing sugars per stem-centimeter and steepens the gradient of sucrose per stem-centimeter (table 6). Ringing also reverses the positive gradient of reducing sugars from leaves to stem in the fourth and fifth segment of the June series. The sucrose gradient from leaves to stem segment is reduced but remains positive.

The positive gradients of acid hydrolyzable carbohydrates and of starch are stable; that is, they are gradients produced by storage, temporary or permanent, of the two groups of substances. These are static gradients brought about by accumulations within living cells or cells associated with living cells. The proportion of living to "dead" cells is much greater in the tops, upper leaves, and the neighboring subterminal stem segment than in the lower portions. The amount of meristematic activity becomes less in proportion to the amount of secondary xylem, cork, and fibers produced toward the basal segment. An important contributory factor to the positive gradients of the static carbohydrates is the increase from the top to the base of the stem in residual dry weight by which the percentages are determined.

[4] MASON and MASKELL (20) defined gradients as positive—high concentrations in the upper and low concentration in the lower portions, and negative—the reverse.

The positive gradient of reducing sugars is for the most part a static one of temporary storage sugars accumulated within living cells and associated with metabolism. Sugars may accumulate against a gradient of reducing sugars. MASKELL and MASON (19) demonstrated that movement of sucrose took place with a positive gradient into the cotton boll, in which the concentration of reducing sugar was much higher than in the stem. Reducing sugars thus accumulated as a storage sugar in the boll. Although in the raspberry experiments bark and wood were not analyzed separately, microchemical data show that much of the reducing sugars contributing to the static gradients are stored in the xylem, pith, and rays.

Part of the positive gradient of reducing sugars is undoubtedly translocatory, as is evidenced by the accumulation of these sugars above the ring (table 5), and the consequent decrease of the gradient (table 6). This is also evidence that the bark, and presumably the phloem, is the region in which the translocatory reducing sugars are moving. The data of tables 5 and 6 are also indicative of the downward movement of reducing sugar.

It remains to explain why, despite a 200 per cent increase in sucrose above the rings, the insignificant sucrose gradient actually increases considerably. It would normally decrease, or if nonexistent would become negative, if sucrose were moving downward in the bark. The evidence for downward movement of sucrose shown by one datum (increase above the rings) seems paradoxical in view of the evidence for upward movement by the other datum (increase below the rings). The canes used in these investigations are annual canes, but they are growing from perennial, sugar-gorged roots to which many other canes supply carbohydrates. If translocation of sugar is considered as polar from a source (the leaves) to a "sink" (the roots), downward movement will proceed along a positive longitudinal gradient (20, 21). The situation is different with the raspberry, however, for there is in the young first year canes apparently no well defined polarized movement. DU SABLON (15) explained the April maximum of carbohydrates in the first year canes of the raspberry as brought about by a migration of carbohydrates from the roots. Thus it is apparent that, in the very early stage of development of the vegetative canes, the usual downward movement

of mobile sugars common to most annuals and perennials is reversed; that is, there is established a temporary root-to-shoot gradient, even though the shoot is highly photosynthetic. During the ensuing growth period the early spring root-to-shoot gradient gradually disappears, owing to the increased production of sugars by the rapidly enlarging foliage region of the cane. Reducing sugars are produced in far greater abundance than sucrose, and the leaves-to-root gradient is soon established. Sucrose is formed in small amounts, and seems to be confined to the bark. The production of an abundance of sucrose in the upper regions of the cane is much slower than the production of an abundance of reducing sugars. During the first season, therefore, the downward movement of sucrose seems to be counterbalanced by an upward movement, seemingly in the same channel, and the net result is a static condition with no apparent gradient. As pointed out previously in this paper, the slight gradients of sucrose, as shown in figures 1 to 4 and in table 6, are thought not to be significant because the gradient exhibited is produced by dividing by a higher residual dry weight in the lower segments than in the upper segments. The gradient apparently does not exist. The effect of a ring on this situation is to reverse the influence of the opposing source of sucrose on the other gradient. The result is an accumulation of sucrose in the segment above and below the isolated segment, and a steepening of the positive gradient above the rings. No data were obtained on the gradient below the rings, but on the basis of the preceding explanation a negative gradient would appear. No analyses were made on the roots.

Usability of residual polysaccharides

Since the introduction by Schulze (**29**) of the term "hemicellulose" to denote a cell wall substance similar in nature to cellulose, ever increasing data point to this group of substances as being of definite physiological significance. Du Sablon (**15**) demonstrated an accumulation of hemicellulose in the fall and a subsequent utilization in the following spring. Schellenberg (**28**) showed that hemicellulose is utilized as a reserve food by trees, and Castoro (**5**) thought that the hemicellulose which he found in the seeds of *Ruscus aculeatus*

functioned as a reserve food. TOTTINGHAM *et al.* (31) assigned to them the function of serving as reserve carbohydrates in apple trees, and ROSA (27) assigned to them a distinct function in the winter hardening process of plants. NIGHTINGALE (25) presented data which indicated that hemicellulose is utilized by the plant as a source of sugar. MURNEEK (24) discussed the significance of hemicellulose as a reserve carbohydrate in apple spurs. CLEMENTS (6) presented convincing evidence that in the sunflower, soy bean, and potato the hemicelluloses are significantly functional in several roles: (1) they serve as temporary reserves under favorable conditions for carbohydrate synthesis; (2) their accumulation and maintenance add to the drought resistance of the plant; (3) they play a morphic role by contributing to the strength of tissues as cell wall thickenings; and (4) they serve as energy reserves in stem and roots. Many investigations have since been reported in which the role of hemicelluloses in drought resistance has been confirmed. Nevertheless LOOMIS, in a report assembled for the committee on chemical methods of the American Society of Plant Physiologists (12), states with regard to the hemicelluloses, "The physiological significance of these materials has not yet been established." Recently CLEMENTS (9), on the basis of evidence in the soy bean, suggested a mechanism of drought resistance in which the gel-forming properties of the hemicelluloses, which not only line the inner walls of the cells but extend into the protoplasm as well, play an important role.

WINKLER and WILLIAMS (32) have recently submitted data on the hemicellulose content of sections of grape stems which they think refute the contentions of the supporters of hemicellulose as a food reserve. They state, in regard to the work of MURNEEK (23), LEONARD (18), and CLEMENTS (6), that "the observed variations of the hemicellulose content were irregular, and furthermore, are not correlated with the vegetative condition of the plant. Their variations certainly do not point to hemicellulose as a reserve." However, these workers did find an unmistakable correlation of hemicellulose fluctuations with the vegetative condition of the plant (6, 9, 10). WINKLER and WILLIAMS found no significant variations in hemicellulose content of grape stems. They showed no data for the other carbohydrate fractions.

In all the work just mentioned the hemicelluloses were determined following acid hydrolysis of not more than six hours' duration. In this investigation the term acid hydrolyzable carbohydrates has been substituted for hemicellulose; furthermore, hydrolysis was carried out for fifteen hours. The quantities of substances extracted in this manner showed large variations in different parts of the plant, attesting their possible role as temporary food reserves.

The hemicelluloses have thus been established as playing a significant physiological role in metabolism. As such they are considered the most stable of the important carbohydrates which are actively associated with metabolism. Their function in metabolism is variable among plants, and depends to a great extent upon the kinds and amounts of other more readily utilizable carbohydrates. Their role may be a minor one in the normal life of some plants under good conditions for growth (**32**), but a change in conditions of growth will usually find its effect in the hemicelluloses. Soy beans react more favorably to conditions of drought than do potatoes and sunflowers, owing primarily to a greater abundance of hemicelluloses (**9, 10**).

Because they are a heterogeneous group of polysaccharides and their derivatives, it is extremely difficult accurately to define their chemical composition. It is likely that this group includes much of the "intussusception" material of the cell walls. The less dehydrated material is hydrolyzed first and in greatest amount. This is the material which is most available as hemicellulose reserve, and it is commonly obtained within a three hour period of hydrolysis. Hydrolysis for a fifteen hour period should yield most of the hemicellulose available for utilization in metabolism.

The total carbohydrates, which in this work included the substances hydrolyzable in 2.5 per cent acid in fifteen hours, were subtracted from the dry weight of the sample, and the residual dry weight was obtained as suggested by MASON and MASKELL (**20**). The residual dry weight consists of minerals (about 3–4 per cent of the dry weight, **7**), fats, total nitrogen, and the stable cell wall material, including cellulose, which is not hydrolyzed in fifteen hours. The total organic nitrogen makes up 1–3 per cent of the residual dry weight, and fluctuates within this range. These substances—fats, minerals, and total organic nitrogen—do not at most account for

more than 5 per cent of the fluctuations in residual dry weight. In the ringed canes the latter deviates as much as 63 per cent of the normal (table 7). The great fluctuations in residual dry weight are therefore owing to fluctuations in residual polysaccharides.

The distributions, in grams per sample, of the residual substances in the first four segments of normal and ringed canes of the June and August series are given in table 7. Included in the table for comparison with the distributions of the June and August series are the figures for the May series. The deviations from the normal of the residual substances in the ringed canes are given in percentages. In the normal canes the residual substances, like the carbohydrates, are distributed in decreasing weights from the base toward the top. The weight gradient in grams is therefore opposite (negative) to the concentration gradient. In the first two segments the residual dry weight of the normal canes remained fairly constant throughout the season.

In the ringed canes the fluctuations of residual substances are in the same direction as those of the total carbohydrates. Where there is a loss of carbohydrates (see May and July figures, table 7), there is a loss of residual substances; and where there is an accumulation of carbohydrates there is a large increase in residual substances. An inversion of the residual polysaccharides takes place as the amount of carbohydrates is depleted by respiration. Above the rings the accumulation of total carbohydrates is faster than the condensation to the residual polysaccharides, owing to the influx of soluble forms from the foliage region. The evident responses of the residual polysaccharides to fluctuations in the total carbohydrates are definite indications of the equilibrium between these two groups. If a sufficiently large shift is produced by depletion or accumulation of the soluble forms, this shift will ultimately produce its effect in the residual polysaccharides. There are no data available which indicate how quickly the adjustment occurs, but the results presented here leave little doubt that it does take place.

COORDINATION OF RESULTS

In the normal distribution of the soluble carbohydrates the reducing sugars are far more abundant than sucrose. This abundance is

superseded only by the acid hydrolyzable substances whose activity seems to be closely associated with the reducing sugars. Their persistent abundance, coupled with the positive gradient of reducing sugars, is associated with the gradient of living cells. This leaves no doubt that the reducing sugars are the major immediate source of metabolic energy, as well as a temporary storage form. Removal of a ring of bark causes an accumulation of the reducing sugars above it, and in so doing decreases the gradient in the stem. These results indicate that a portion of the reducing sugars is functional in translocation in the bark. The positive gradient of reducing sugars in the normal canes is the sum of two components: (1) a static positive gradient of temporary storage sugars associated with active protoplasm; and (2) a dynamic positive gradient of reducing sugars which are translocatory.

The concentration of sucrose is very low at all times of the season. However, it is by far the most responsive to ringing of all forms, and this sensitivity is evidence of its role in the metabolism of the plant. Sucrose apparently is not produced in the raspberry by a fructose-glucose concentration maximum. Its gradient, if it has one, is so slight that it is well within the error produced by variations of the residual dry weight upon which the concentrations are expressed. It is thought that no gradient exists. Ringing causes a 200 per cent (of the normal) accumulation above and below the rings, and a 100 per cent decrease between the rings. The slight positive gradient which might exist is made more positive; if the gradient does not normally exist, a positive one appears. These data are interpreted to mean that:

1. Sucrose serves essentially as a translocatory form.

2. It moves in the bark, probably in the phloem.

3. There is an upward movement of sucrose from the perennial roots into the young shoot. It is probable that some of this sucrose is supplied by normal canes growing from the same roots.

4. As the developing cane enlarges and produces an increasingly greater photosynthetic area, sucrose is formed in the upper region of the cane and tends to move downward with its own gradient.

5. During the growth of the first year canes there is a period of adjustment of the two sources of sucrose. The gradient of one is

compensated by that of the other, and no gradient appears. It is likely that in the second year a definite positive gradient of sucrose appears, owing to the preponderance of the sugars produced by a large photosynthetic cane.

6. Ringing removes the influence of one source of sucrose upon the other, and an accumulation occurs above and below the isolated segment. A gradient, which before was compensated by one issuing from an opposing source, is made to appear.

It is significant that sucrose entirely disappears from the segment between the rings, whereas the reducing sugars are always present, although low in quantity. Unlike the reducing sugars, sucrose does not appear upon hydrolysis of the "hemicelluloses"; therefore sucrose is not a product of hydrolysis. Perhaps it is formed, in a manner similar to that found in the cotton plant by PHILLIS and MASON (26), within the sheath cells or companion cells of the phloem, and appears essentially in the phloem. In the raspberry it seems to have no function other than one of translocation.

Starch plays an insignificant role in the metabolism of the raspberry. BRIERLEY and LANDON (4) were unable to demonstrate microchemically the presence of starch, even in the fruiting canes. The acid hydrolyzable substances were shown to be intimately associated with the reducing sugars, being in equilibrium with them and fluctuating in the same direction. When the fluctuations in reducing sugars are sufficiently great, not only do the acid hydrolyzable substances respond, but also the residual polysaccharides. The latter are thought to include the remaining portion of the "intussusception" material of the cell wall which is not taken out in the acid hydrolyzable fraction.

Thus it appears that all carbohydrates, with the probable exception of the primary cellulose wall, are actively or potentially functional in the metabolism of the plant. The "exceedingly complex dynamic equilibrium" of which SPOEHR (30) writes extends beyond the hemicelluloses to include the cell wall polysaccharides and their derivatives. The activity of this group in carbohydrate metabolism depends upon: the kind of plant; the amounts of other carbohydrates present; the nature and stability of the polysaccharides and

derivatives making up the hemicelluloses; the residual substances; and, what is still more important, the magnitude of the shift in the "dynamic equilibrium."

Summary

1. The seasonal fluctuations in reducing sugars, sucrose, starch, acid hydrolyzable carbohydrates, total carbohydrates, and the residual polysaccharides are recorded for the raspberry plant.

2. Double rings, one 15 cm. and another 30 cm. above the ground, isolating a 15 cm. segment of stem, were used to check movements of sugars.

3. In normal plants the concentration of total carbohydrates is fairly high in May, drops during June, the period of most active growth, and then increases to a maximum in August. The acid hydrolyzable carbohydrates are the largest fraction of the total carbohydrates. The concentration of reducing sugars is highest in May, drops rapidly in June, and increases slightly in July and August. A low concentration of sucrose is present at all times. Starch is never abundant, and apparently is not an important carbohydrate reserve.

4. In ringed plants the total carbohydrates accumulate above the rings, and decrease below and between them. Sucrose made the largest increase above the ring. Reducing sugars accumulated above the rings, but decreased between and below them. Apparently sucrose tends to move upward as well as downward in the bark. It does not appear to be a product of hydrolysis of hemicellulose in the isolated segment as do reducing sugars. A portion of the reducing sugars is translocatory, moving downward in the bark.

5. All carbohydrate gradients are positive. Sugars move downward with a marked positive gradient. The positive gradients of the acid hydrolyzable substances and of starch are static. These gradients are produced by temporary storage brought about by accumulations within living cells and cells associated with living cells. Ringing decreases the positive gradients of reducing sugars, indicating that some of them are mobile in the bark along a dynamic positive gradient. Much of the reducing sugars may be a temporary storage

form and not functional in translocation. Therefore their positive gradient is the result of a positive dynamic gradient of mobile sugars and a positive static gradient of temporary storage sugars.

6. The insignificant and probably non-existent gradient of sucrose is increased rather than decreased, as would be expected if this sugar were moving only downward.

7. The residual substances consist of those polysaccharides, small quantities of fats, minerals, and organic nitrogen which remain after the total carbohydrates, including acid hydrolyzable substances, are subtracted from the dry weight. The residual polysaccharides are thought to include the "intussusception" material (polysaccharides of high molecular weight and their derivatives) of the cell wall. The residual polysaccharides fluctuate as the total carbohydrates (particularly reducing sugars and the "hemicelluloses") fluctuate, and in the same direction.

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EXPERIMENTS ON THE TRANSPORT OF AUXIN[1]

F. W. WENT AND RALPH WHITE

(WITH TEN FIGURES)

Introduction

The purpose of the work described in this paper was to measure quantitatively the transport of auxin, using a new experimental technique. The original method of measuring the rate of auxin transport required the determination of the amount transported through the tissue to an agar block during a series of time intervals, thus determining the time at which the first auxin arrived (**6**). Since with the photokymograph (**4**) the beginning of the curvature can be accurately determined, and since this initial curvature occurs a known time interval after application of auxin to the recording plant, it is possible to determine for each coleoptile when auxin reaches its cut surface. Thus by placing a piece of tissue one-sidedly on a recording plant, and applying auxin to the other end of this tissue, the rate of auxin movement through it can be determined.

Method

Preliminary experiments were all unsuccessful for one of two reasons. When coleoptile sections were interposed between the recording coleoptile and the agar block containing auxin, either the curvatures started simultaneously with the controls as if no section were present, or no curvatures appeared at all. Finally, however, the technical difficulties were overcome, and transport velocities of the same order of magnitude as VAN DER WEIJ (**6**) described were obtained. To this end the following improvements in technique had to be made, for the rest adhering to the procedure previously outlined (**4**). Figure 1 gives a sketch of the new set-up.

The grass peduncle inserted in place of the primary leaf did not give a satisfactory support of the applied tissue plus agar block, and

[1] This work was carried out with the aid of the Works Progress Administration, Official Project no. 165-03-6999, Work Project no. 6330-6989.

the latter often stuck to it. The method by which the leaf is partly pulled out and a fine metal wire (*D*) is inserted gave good results, provided the agar block (*A*) and the top of the transport tissue (*B*) did not touch the wire. It was also found that copper wire gave irregular results when not properly inserted and touching the coleoptile walls; nichrome wire and especially silver wire nr. 36 (diameter 0.12 mm.) were very satisfactory.

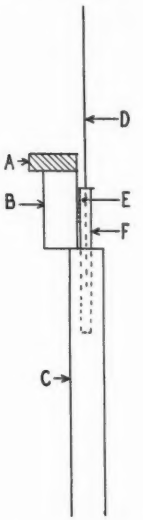

FIG. 1.—Schematic picture of test plant *C*, with silver wire *D* inserted in the partly pulled out primary leaf *F*. On one side of its cut surface the transport section *B* is stuck by means of petroleum jelly *E* against the primary leaf *F;* on top is an agar block *A*, containing the substance to be transported.

The primary leaf (*F*), after pulling loose, was cut off so that the transport tissue would protrude about 2 mm. above it. A thin film of petroleum jelly (*E*) was applied to the upper part of the protruding part of the primary leaf, care being taken not to come in contact with the cut surface of the coleoptile as it would interfere with the transport of auxin. The transport tissue (*B*) was then placed on the cut surface of the recording coleoptile (*C*) and held in place by the petroleum jelly (*E*) against the primary leaf (*F*). The contact between the tissues is very essential and was therefore inspected with a magnifying glass. The agar blocks (*A*) containing the growth promoting substances were then placed on top of the transport tissue, so that they would not touch the wire and the contact between transport tissue and recording plant would not be broken. In all experiments, blocks of 2 × 2 × 1 mm. 2 per cent agar were used, which were washed in the solutions for 1–2 hours.

To remove the auxin still contained in the tissues through which transport had to be measured, and for which mostly *Avena* coleoptile sections were used, these were placed with their basal cut surface on wet filter paper for 3 hours. After that they did not induce curvatures by themselves when no auxin was applied.

The most important departure from the standard conditions as described by SCHNEIDER and WENT (4), however, was by decreasing the relative humidity of the air to 75–77 per cent during the test. This unfortunately causes premature drying of the agar blocks, but for the first 3 hours the decrease in volume is not considerable. This lowering in humidity is essential to prevent leakage of auxin along the surface of the transport tissue. That leakage actually occurred at high humidities was found by placing inverted coleoptile sections on the recording plants and applying high concentrations of indoleacetic acid in agar on them. When this was done at 85–90 per cent humidity strong curvatures resulted; at 76 per cent humidity there was no curvature at all, and therefore no transport through the inverted sections. In normal sections, of course, considerable transport took place. At 88 per cent humidity lanolin paste containing 0.1 per cent indoleacetic acid gave no inverse auxin transport through 6 mm. coleoptile sections, since no auxin would leach from the paste. Further details of this polar transport are given later in this paper.

For the transport test, the recording plants (as well as the sections) were even more carefully selected than in the standard *Avena* test. Among other factors, this selection was for thick coleoptile walls, which gave a better contact surface. Plants 30 mm. long were found best.

In general the reaction of the recording plants was delayed, owing to the extra time required for the auxin to reach the plant through the transport tissue. Since in the *Avena* test plants, sensitized through a double decapitation, the "regeneration" opposes the auxin curvature after 80 minutes, the standard double decapitation method (10) was not suitable for this transport test. Therefore the coleoptiles were decapitated only once, and the transport tissue with auxin was applied immediately, so that the curvature could occur before the regeneration (4, p. 477). To make the control plants comparable, the agar blocks without transport tissue were applied 40 minutes (mean transport time) after decapitation.

Evaluation of results

All these transport experiments have been repeated several times; the reaction of a total of more than 2000 plants has been recorded in

all. Only about one-sixth of this material is here presented, since all results were closely similar. Some of this additional material, however, is incorporated in tables 2 and 3.

Before the experiments are discussed in detail, certain difficulties in the interpretation of the data should be pointed out.

On account of the variability of the material, some method of averaging is essential. As previously described (4), the position of

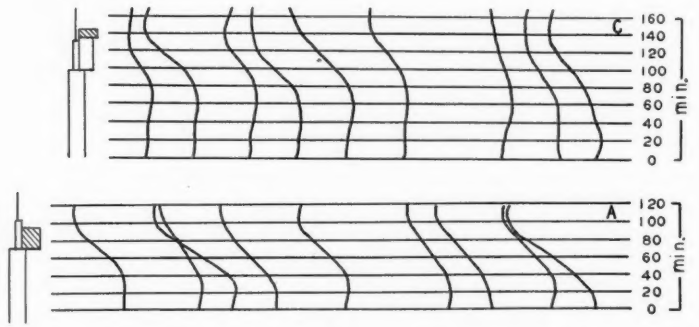

FIG. 2.—Retracing of part of record of experiment represented in fig. 3. Parallel lines drawn at 20 min. intervals, extending between them. Where they are perpendicular to the time lines the plants remain straight; slope toward left means negative (growth) curvature. Curve *A:* controls, curvatures start 20–40 min. after first recording. Time elapsed between putting on of agar block (containing 0.1 mg./l. indoleacetic acid) and first recording, 4 minutes. Curve *C:* 6.3 mm. coleoptile section inserted between agar block (containing 4 mg./l. indoleacetic acid) and test plant. Curvatures start 60–80 min. after first recording, which occurred 8 minutes after putting on the agar blocks.

the tip of the test plant is recorded once every 4 minutes. As long as the plant does not curve, this position remains the same, and a straight line is recorded (fig. 2). Although in general the onset of the curvature is very sudden, so that it should not be difficult to determine in which 4 minute period the curvature starts, yet unfortunately the nutations which are always present to some extent make it difficult to measure this point with the desired accuracy. When the rate of curvature is small, it is also difficult to tell when the curvature started. By taking the mean of the reactions of eight to ten plants, these nutations are ruled out and the onset of the curva-

ture can be determined with greater accuracy from the mean curve, as shown in figures 3–10.

But a new difficulty arises: in these mean curves the beginning of the curvature will not be the mean of the onset of the individual plants, but will depend upon the moment that the first one starts to bend. Therefore there must be a systematic difference between the two types of calculation (table 1). Figure 2 is a retracing of the record of the experiment represented in figure 3, curves *A* and *C*.

TABLE 1

TIME BETWEEN APPLICATION OF AUXIN AND (1) ONSET OF CURVATURE OR (2) INTERSECTION POINT, MEASURED FOR INDIVIDUAL TEST PLANTS AND CALCULATED FROM AVERAGE CURVES OF FIGURE 3. FOR A DESCRIPTION OF INDIVIDUAL TREATMENTS (*A*, *B*, *C*, *D*), SEE FIGURE 3

CURVE	TYPE OF READING	INDIVIDUAL TIMES IN MINUTES (MEASURED FROM FIGURE 2)	MEAN	CALCULATED FROM FIG. 3	MEAN OF THREE SHORTEST TIMES
A.....	Onset	20, 28, 36, 32, 32, 32, 28, 28, 44 ..	31	26	28
	Intersection	34, 42, 44, 38, 46, 38, 42, 40, 52 ..	42	41	37
B.....	Onset	38, 42, 30, 46, 34, 34, 34, 42, 46 ..	38	32	33
	Intersection	36, .., 44, 46, 48, 32, 38, 58, 46 ..	43	40	35
C.....	Onset	76, 68, 72, 60, 72, 80, 76, 80	73	67	67
	Intersection	80, 86, 96, 84, 84, 94, 96, 94	89	87	83
D.....	Onset	60, 64, 56, 72, 64, 60, 56, 80, 64, 64	64	55	57
	Intersection	62, 76, 72, 74, 76, 78, 74, 80, 86 ..	75	73	69

The individual reactions are all similar, and in no single case can there be doubt that the transport section greatly delayed the onset of the curvature. For the rest the type of reaction is the same. After a short start phase, the rate of curvature becomes constant (eumotoric phase), remains so for 30 to 60 minutes, and then decreases. The eumotoric phase can be used for an unambiguous determination of the beginning of the curvature. If a line is drawn extending this eumotoric phase it will cross the line which extends the original position of the plant, and the intersection between the two lines is easy to determine and is a much more definite point than the onset of the curvature (points *A* and *D*, fig. 3). This point also depends

on the moment of arrival of the auxin at the cut surface. But the mean of the individual intersections does not significantly differ from the intersection derived from the average curve (table 1). Besides, this point of intersection is more or less independent of the auxin concentration. This is not true of the time of onset of curvature, which can be detected the sooner the greater the rate of curva-

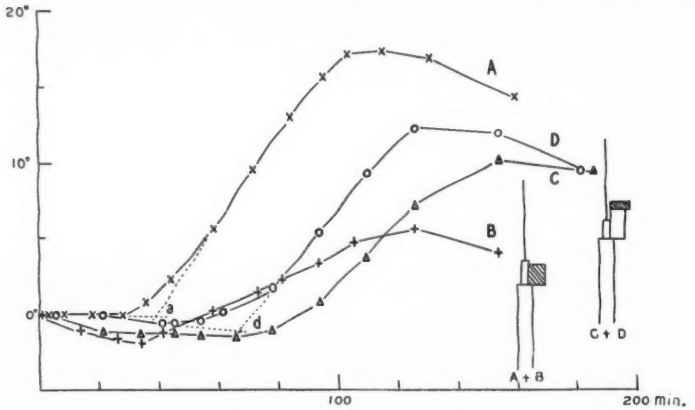

FIG. 3.—Abscissa: time in minutes after application of auxin. Ordinate: movement of tip of test plants in degrees. Curves *A:* 0.1 mg./l. indoleacetic, applied directly; *B:* 0.05 mg./l. indoleacetic acid, applied directly; *C:* 4 mg./l. indoleacetic acid, applied on intervening 6.3 mm. upright coleoptile section; *D:* as *C*, but concentration 2 mg./l. indoleacetic acid.

ture (= rate of auxin arrival; table 1). These considerations sufficiently justify the use of this intersection point throughout this paper.

BASIC EXPERIMENTS

Figure 3 shows the course of the curvature of the test plants when agar blocks containing auxin were placed on directly, or with an intervening coleoptile section of 6 mm. Each curve is calculated as the mean of eight to ten test plants. Figure 2 and table 1 give data on the individual reactions in this experiment. A few conclusions, substantiated by other experiments which will not be described here, can be drawn from figure 3. In the first place it is seen that the slope of the curve (that is, the rate of curvature) depends upon the con-

centration of the auxin in the agar block (curves *A* and *B*; see also curves *A* and *B*, fig. 6). This is the same as described before (4, p. 488). There is no difference in the rate of curvature of the test plants carrying the transport sections (curves *C* and *D*), indicating that they represent "maximum angles"; that is, the auxin concentration reaching the test plants was so high that they curved at a

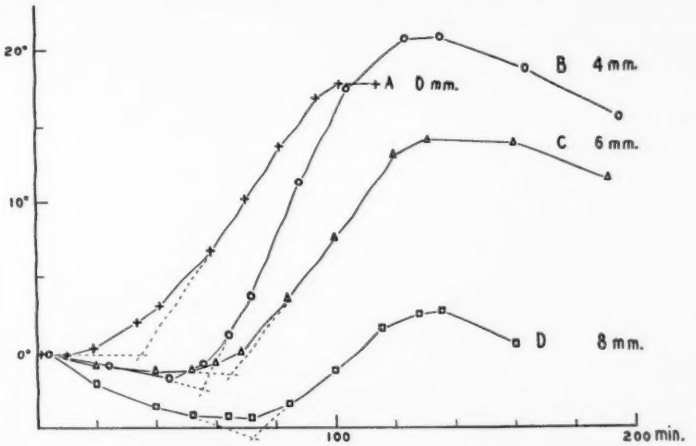

FIG. 4.—Abscissa: time in minutes after application of indoleacetic acid 0.1 mg./l. (curve *A*) and 2 mg./l. (curves *B*, *C*, *D*). Ordinate: movement of tip of test plants, in degrees. Curves *A:* agar directly applied to test plant; *B:* applied on 4.2 mm. transport section; *C:* on 6.3 mm.; *D:* on 8.4 mm.

maximal rate. The type of the curves *A*, *C*, and *D* is similar, with the exception of the moment when the plants start to curve. The transport time of auxin through 6.3 mm. sections was 39.5 minutes in this experiment, corresponding to 9.6 mm./hour.

The experiment of figure 4 compares well with that of figure 3. Each curve was calculated as the mean of eight to ten test plants. The transport time through 4.2 mm. sections (curve *B*) was 55–35 = 20 minutes, for 6.3 mm. sections (*C*) 65–35 = 30 minutes, and for 8.4 mm. sections (*D*) 77–35 = 42 minutes. Thus the conclusion of VAN DER WEIJ (6) is confirmed, that the velocity of transport is independent of the length of the coleoptile section, in this experi-

ment being 12.4 mm./hour. The amount transported, however, decreases with the longer sections.

Figure 5 shows the auxin transport through sections cut from different levels of the coleoptile. In agreement with the experiments of VAN DER WEIJ (6) with transport of an auxin concentrate from urine, it was found that the sections cut nearest the tip (*B*) transported somewhat more auxin than middle sections (*C*), but basal

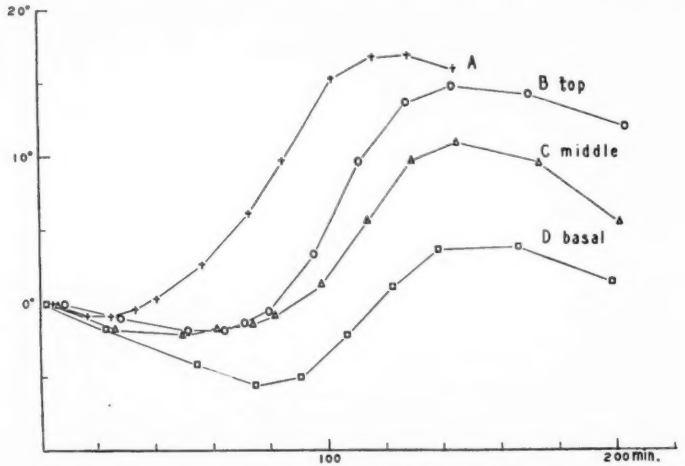

FIG. 5.—Abscissa: time in minutes after application of indoleacetic acid 0.1 mg./l. (curve *A*) and 2 mg./l. (curves *B*, *C*, *D*). Ordinate: movement of test plants in degrees. Curves *A:* control, agar directly applied to test plant; *B*, *C*, *D:* with interposition of 6.3 mm. coleoptile sections (*B*, cut 3–9 mm. from tip, *C* 9–15 mm., and *D* 16–22 mm.).

sections gave distinctly less transport (*D*). The velocity differed very little in the three regions of the coleoptile. Since VAN DER WEIJ did not find differences in transport capacity at various heights in the coleoptile, when *Zea* tip auxin was used, it would indicate that his auxin concentrate resembled the indoleacetic acid which we used in our experiments rather than the auxin *a* or *b* from *Zea* coleoptile tips. These experiments indicate that never more than the upper 9 mm. of the decapitated coleoptile should be used for transport sections.

Since only a small fraction of the cut surface of the transport sec-

tion is in contact with the test plant, it was of importance to reinvestigate the auxin transport through the tissues containing the vascular bundles, and through the parenchyma. On the cross section the coleoptile is elliptical, and the two vascular bundles extend along the narrow side. In two experiments, one of which is summarized in figure 6, the transport sections were attached to the test

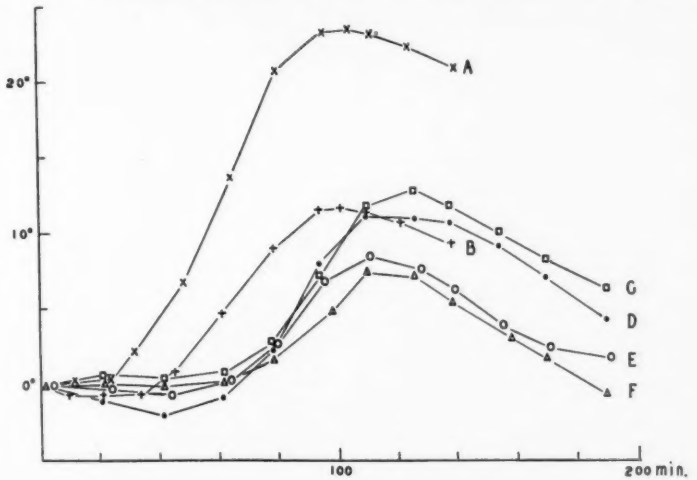

FIG. 6.—Abscissa: time in minutes after application of indoleacetic acid 0.25 mg./l. (curves *A*, *C*, *D*) and 0.1 mg./l. (curves *B*, *E*, *F*). Ordinate: curvature of test plants in degrees. Curves *A* and *B:* controls, agar directly applied to plant. Curves *C–F:* 4.2 mm. coleoptile section between agar and test plant, in *C* and *E:* vascular bundle of transport section in contact with test plant, *D* and *F*, parenchyma only.

plants either with their broad (*D* and *F*) or with their narrow (*C* and *E*) sides. Both experiments gave identical results, showing that the auxin transport is practically independent of the position of the transport section; in other words, that the presence of the vascular bundle does not materially increase the auxin transport. This is in agreement with the results of VAN DER WEIJ (**6**) and WENT and THIMANN (**10**), but differs from the conclusions of LAIBACH and KORNMANN (**3**). Although no difference was found in regard to the position of the transport sections, still in all experiments care was taken to place them with their narrow side against the test plant.

Polarity of transport

There were many reasons for investigating the polarity of auxin transport with the new technique. In the most accurate transport experiments (**6, 7**) the mean transport of twelve coleoptile sections was always determined, so that occasional leakage through one or more sections could not be detected. Besides, as mentioned under Method, the previous experiments on auxin transport in the *Avena* coleoptile were all carried out at a high humidity, which al-

TABLE 2

Concentrations of indoleacetic acid applied dissolved in agar or in lanolin, tested with respect to their ability to move from base to apex through Avena coleoptile sections of differing length

Length of coleoptile section (mm.)	No inverse transport with concentratigns		Inverse transport with concentrations	
	mg./l. sclution in agar	mg./gm. lanolin	mg./l. solution	mg./gm. lanolin
2.1	.....	.....	.....	10
3.1	50, 200	2, 1	1000	5?, 10
4.2	100	10	1000	.....
6.3	2, 4, 10, 50, 200, 1000	1	.....	.....
10.5	4	.....	.....	.....

lows a certain amount of auxin leakage along the surface of the coleoptile sections. This leakage must have been considerable, especially in Jost and Reiss' (**2**) experiments, since their coleoptiles were made to adhere to the wall of the glass vessel by capillary action of the solutions containing auxin. Thus it is not surprising that they failed to obtain clear evidence for polar auxin transport. Also it would now seem that part of the inverse transport reported by Went (**9**) might have been due to leakage along the surface of the coleoptiles, since the transport experiments were carried out in closed petri dishes in a high humidity. However, the falling off of the transport beyond concentrations of 1 mg. indoleacetic acid/cc. is proof that not all of the inverse transport was due to leakage.

At humidities above 80 per cent, applying auxin in agar, no transport tests showed either polarity of the auxin movement

through the tissues or an appreciable time lag of the auxin curvature when a coleoptile section even in normal position was interposed between agar block and test plant. However, when not auxin agar but auxin paste (1 mg. indoleacetic acid per gram lanolin) was used, even

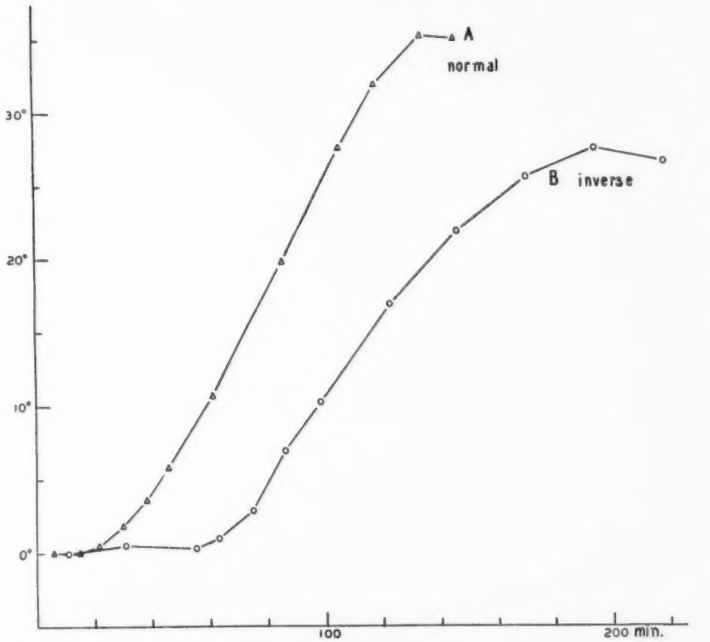

FIG. 7.—Abscissa: time in minutes after application of indoleacetic acid 0.5 mg./l. (curve *A*) and 1000 mg./l. (curve *B*). Ordinate: curvature of test plants in degrees. In curve *A*, a 3.1 mm. coleoptile section was placed apex up between test plant and agar; in curve *B* this section was placed apex downward (inverse transport).

at a humidity of 85–90 per cent no trace of leakage or non-polar transport was observed.

Since auxin concentrations are physiologically better defined in agar than in lanolin, most transport experiments were carried out with auxin-agar at humidities of 70–80 per cent, with occasional checks with auxin-lanolin pastes. Table 2 summarizes the experiments on inverse auxin transport (each determination is the mean of

ten to twenty test plants). In all cases control determinations were made with coleoptile sections in the normal position, and in every case good transport of a 0.5, 1, or 2 mg./l. solution of indoleacetic acid was obtained, indicating that the lack of transport through the inverted sections was not due to unfavorable experimental conditions. The data in table 2 leave no doubt that the longer the sec-

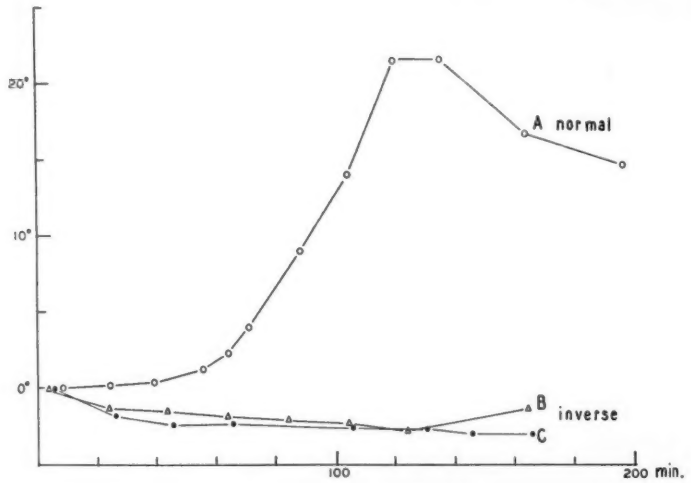

FIG. 8.—As fig. 7, but length of coleoptile section 6.3 mm. Curves *A:* indoleacetic acid 1 mg./l., section apex upward; *B:* indoleacetic acid 200 mg./l., section apex downward; *C:* indoleacetic acid 1000 mg./l., section apex downward.

tions the more strictly polar is the transport. Whereas the 3.1 and 4.2 mm. sections still permit the passage of 1000 mg./l. indoleacetic acid solutions, the 6.3 mm. sections do not do so any more. Figures 7 and 8 give the results of two actual experiments. In each case it was observed that the velocity of inverse transport was considerably less than of normal transport (4–6 mm./hour against about 10 mm./hour), whereas the capacity was greatly less, especially when the enormously increased concentration gradient is considered. Thus the inverse transport differs from the normal transport in more than one way.

Transport of different compounds

With the described test it is possible to measure the velocity and capacity of transport through coleoptile tissues of all substances active in the *Avena* test. This has been done with indole(3)acetic acid (figs. 3–8), indole(3)butyric acid, anthraceneacetic acid (fig. 9), naphthaleneacetic acid (fig. 10), indole(3)propionic acid, and

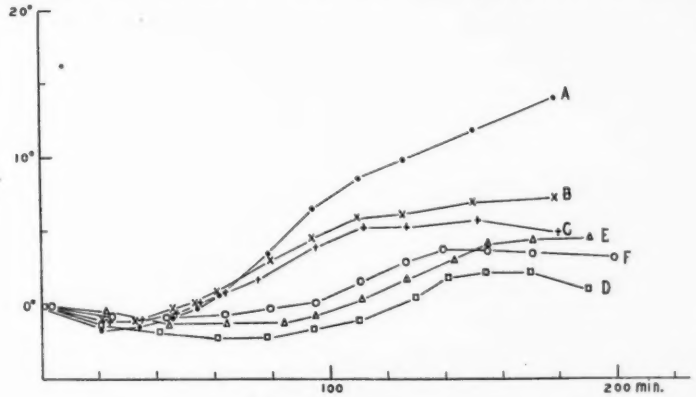

Fig. 9.—Abscissa: time in minutes after application of anthraceneacetic acid. Curves *A–C*, no sections; *D–F*, 4.2 mm. sections between agar and test plant. *A:* concentration 4 mg./l.; *B:* 2 mg./l.; *C:* 1 mg./l.; *D:* 100 mg./l.; *E:* 50 mg./l.; *F:* 20 mg./l. Ordinate: curvature of test plants in degrees.

cis-cinnamic acid. The last two did not show any transport at all under the conditions described, and will not be considered further. This was not due to a lack of response of the test plants, since these substances gave curvatures in the standard *Avena* test. In the first four compounds mentioned, however, good values for transport velocity were obtained, and these are given in table 3. The figures in this table are means of three to ten separate determinations with ten plants each.

The figures of line *A* are calculated from the controls in each experiment, and indicate the time required for the beginning of the curvatures after application of the agar containing the active substance. These figures give an indication of the velocity of the growth reaction in which these compounds must take part.

The second line (*B*) is the reciprocal value of the transport velocity of these compounds. It will be seen that, although there is great variation in the individual determinations, still there is no doubt that the transport of naphthaleneacetic acid is considerably slower than that of indoleacetic acid. This is not due to differences in the applied concentrations, since figures 3, 6, 9, and 10 show that there is no systematic difference between the transport velocities, or the moments of incipient curvature in the controls, when different concentrations are tested.

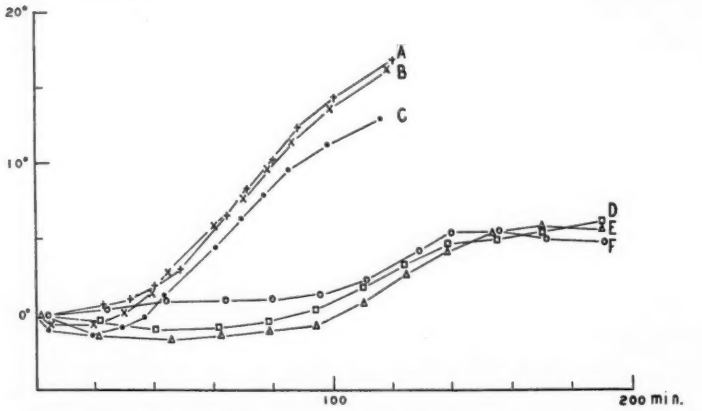

FIG. 10.—Abscissa: time in minutes after application of naphthaleneacetic acid. Curves *A–C:* agar applied directly; *D–F:* 4.2 mm. coleoptile sections between agar and test plants. *A:* concentration 4 mg./l.; *B:* 2 mg./l.; *C:* 1 mg./l.; *D:* 50 mg./l.; *E:* 20 mg./l.; *F:* 10 mg./l. Ordinate: curvature of test plants in degrees.

Line *D* is a summary of results obtained with the standard *Avena* test, and indicates the length of the curved zone 90 minutes after one-sided application of the various compounds. Also in these experiments no differences in length of curvature were observed if different concentrations were applied, which also indicates that transport velocity is independent of concentration. VAN DER WEIJ (6) has come to the same conclusion.

It is interesting to calculate with the aid of lines *A* and *B* how long the curved zone in an *Avena* test would be. Since the transport experiments were carried out with *Avena* plants which were decapitated only once, which retards the onset of the curvature 10 to 20

minutes (4, p. 477), and since the apparent transport is slowed down owing to two extra cut surfaces, the calculations were made for a reaction time of 118 minutes. The calculated values obtained in this way (line *C*) are in good agreement with the actually measured length of the curved line in the standard *Avena* test (line *D*).

There is another point which is of interest. Applied indoleacetic acid may give a maximal curvature, even when it reaches the test plant through a transport section, indicating that the amount

TABLE 3

RATE OF GROWTH REACTION (A); TRANSPORT RATE (B); LENGTH OF CURVED ZONE AS CALCULATED FROM A AND B (C); AS DETERMINED IN STANDARD AVENA TEST (D); AND ACTIVITY OF FOUR GROWTH PROMOTING SUBSTANCES IN AVENA COLEOPTILES

	Indole(3)acetic acid	Indole(3)butyric acid	Anthracene-acetic acid	Naphthalene-acetic acid
A. Time required for beginning of curvature in controls..	37 min.	53 min.	47 min.	34 min.
B. Time required for transport through 4.2 mm. sections..	28± 1.9 min.	38±5.3 min.	47±1.8 min.	66±3.8 min.
C. Calculated length of curved zone after 118 minutes...	12.1 mm.	7.2 mm.	6.3 mm.	5.4 mm.
D. Length of curved zone in standard Avena test.....	11.8 mm.	7.8 mm.	5.5 mm.	6.0 mm.
Concentration giving 5° curvature in standard Avena test	0.05 mg./l.	1 mg./l.	1 mg./l.	2.5 mg./l.

transported is considerable. From figure 6 it would appear that almost as much indoleacetic acid reaches the test plant through the interposed tissue as from the agar block directly. This is not true for indolebutyric acid, naphthaleneacetic acid, and anthraceneacetic acid. At concentrations of 10–20 mg./l. practically as much active material reaches the test plant through the transport section as at a five times higher concentration. But the controls indicate that the plants are able to give a much larger reaction. Thus it is clear that, for those substances, the amount transported (or the transport capacity) is limited.

TRANSPORT THROUGH DIFFERENT TISSUES

The method described in this paper can be used to measure the auxin transport through other plant tissues also. Only they should be small enough so that they can be fitted on an *Avena* coleoptile. In a few cases very rapid transport was obtained through *Nitella* internodal cells.

Small parallelepipeds were cut from potato tubers in longitudinal and radial direction, but in no case was any transport found, neither of 10 nor 2 mg. indoleacetic acid per gram lanolin.

In corn coleoptiles good transport was found, but only when high concentrations were applied (10 mg. indoleacetic acid per gram lanolin). In one experiment the transport of low auxin concentrations through 4.2 mm. *Avena* coleoptile sections gave maximum angles, with 59 and 62 minute intersection points in two sets of ten plants each, and no inverse transport occurred. In the 4.2 mm. corn coleoptiles only small curvatures resulted with high auxin concentrations, intersection point 87 minutes (eight plants). Also inverse transport of this same concentration took place, intersection point 102 minutes (ten plants).

Through 4.2 mm. papaya leaf stalks and midribs only very little was transported (120, 128, and 132 minute intersection points), but good results were obtained with 3.1 mm. sections of the same material.

Through young hypocotyls of tobacco plants no transport was observed with 1 mg. indoleacetic acid per gram lanolin, but the same concentration very clearly moved only from apex to base through *Tropaeolum* leaf stalks and flower stalks.

Thus this technique allows the determination of auxin transport through many different types of tissues. It is interesting to note that in general the velocity and capacity of transport were much less than in *Avena* coleoptiles. This work will be continued.

Discussion

It is not necessary to repeat the discussion on polarity of the auxin transport, as given by WENT and THIMANN (10). The present paper gives additional and rigid proof of the almost absolute polarity of the *Avena* coleoptile tissue for the transport of indoleacetic acid. It

also indicates why some investigators, especially JOST and REISS (2), did not observe this polarity, since it is apparent only when leakage along surface films is small or excluded. Of course it is still possible that the inverse transport of the highest auxin concentrations through 3 and 4 mm. sections was due to some leakage. This does not seem likely, however, considering the long transport time and the sudden onset of the curvature.

With regard to the remarkable differences in the amount of auxin leakage between the older transport experiments with the original technique (**8, 6, 9**) and these new experiments, something more has to be said. Whenever the auxin moves to a recipient in the form of an agar block, diffusion through tissues or the agar greatly exceeds leakage along the surface. The latter must occur of necessity, since the auxins are surface active; that is to say, they will accumulate in a water-air interphase. How relatively unimportant a movement along this interphase is in competition with diffusion is demonstrated by the value obtained for the diffusion constant of auxin. This was determined by letting the substance diffuse from a thin agar layer into a stack of three other agar layers. The value obtained in this way did not differ greatly from the value expected, on account of the molecular volume, so that the spreading along the agar surface could not have been more than a fraction of the total amount diffusing.

To account for the relative importance of surface leakage in the type of experiments described in this paper, the following fact has to be remembered. Although movement of auxin in an interphase will take place in a monomolecular film, it may be exceedingly fast, provided all auxin arriving at one end is immediately removed (1). This will not be the case if at this end the auxin has to diffuse into agar. But if the arriving auxin is completely removed by the apical cells near the cut surface of the coleoptile (which is quite possible since the auxin transport inside the coleoptile is equally large with, as against, a concentration gradient, 7), a large amount of auxin might be moved in the moist surface film along the epidermis of the coleoptile. Thus the discrepancy in the results might be in the type of receiving material of the auxin, whether agar or living plant cells.

The transport rate experiments with different substances have

been the first to allow a critical test of THIMANN's hypothesis (5) that the activity of a substance in the *Avena* test depends not only on its growth activity per se, but also on its transport rate. Thus substances with a slow rate of transport would seem only slightly active in the *Avena* test. As far as this work goes it has borne out THIMANN's assumption. If the substances tested are placed in order of their molar activity in the *Avena* test, indoleacetic > indolebutyric, anthraceneacetic > naphthaleneacetic > indolepropionic > cis-cinnamic acids, then this series represents the order in which the transport rates of these substances decrease. For the last two it could not be measured at all. This is not surprising, since the curvatures they induce in the *Avena* test are shorter than 4 mm.

At the same time it is apparent that the difference in transport velocity is not the only factor which determines the difference in activity of these substances in the *Avena* test. First there is a difference in the time of onset of the curvature, which is independent of transport velocity. Therefore, this moment of beginning of the curvature must be determined by the rate of penetration of the compound into the cell and/or the rate of the growth reaction proper. Especially such growth promoting substances as have a benzene nucleus only (as phenylacetic acid) have a very slow rate of reaction. In addition, the fact must be considered that the capacity of the transport of growth promoting substances is very limited. This means that if less is transported than is necessary for a growth response no curvature at all will result. This is the case with most growth promoting compounds with a benzene nucleus, which all give S-shaped curvatures in the *Avena* test. Take phenylacetic acid as an example. That it is transported downward is indicated by the positive curvatures which it induces down to about 1 cm. below the cut surface, when an agar block containing a 10^{-4} molar solution is put one-sidedly. When sections are submerged in this concentration they will grow as much as they do in the considerably lower optimal indoleacetic acid concentrations. In the *Avena* test, however, the negative curvature due to the growth promoting properties of phenylacetic acid is limited to a zone of not more than 2 mm. length. These facts can be correlated in the following way: To get equal increases in growth, about a 100× higher concentration of phenyl-

acetic than of indoleacetic acid is required. Since the amount which can be transported inside the *Avena* coleoptile is limited, apparently not enough phenylacetic acid reaches the growing cells farther down to produce a visible growth increase, although the positive curvature indicates that some phenylacetic acid was present. In another paper this curious positive curvature will be considered in greater detail.

Summarizing this discussion, it may be said that the extent of the *Avena* test curvature depends on several different properties of a growth promoting substance: (1) the rate of transport of the substance inside the coleoptile tissue; (2) the rate of growth reaction, indicated by the time of beginning of the growth curvature; (3) the amount transported in connection with the activity of the substance in *Avena* growth. Point 3 must be investigated with more direct methods, but until we have a method which will allow the analysis of extremely small amounts of such phenyl compounds, no direct determinations seem possible.

Summary

1. Adapting the photokymograph recording of the *Avena* test, a new technique for measuring the velocity and capacity of the transport of growth promoting substances through different tissues has been worked out. For this the rate of bending and the moment of beginning of curvatures are compared for a normal *Avena* test and one in which the agar block is separated from the test plant through the tissue of which the transport properties are to be determined. Thus for individual pieces of tissue these properties can be calculated.

2. In general the results of VAN DER WEIJ (**6**) were confirmed, as far as the transport properties of the *Avena* coleoptile are concerned. Transport velocity is independent of length of tissue, but the amount transported decreases with increased length of coleoptile section. Transport through the narrow or wide side of a coleoptile is practically the same. The lower part of the coleoptile has slightly different transport properties from the more apical zones.

3. The polarity of indoleacetic acid transport is far more pronounced than most of the earlier investigators found. In our experiments leakage along moist surfaces was more nearly excluded. Only

the highest auxin concentrations (1000 mg./l.) were transported from base toward top through 3.1 and 4.2 mm. sections, but not through 6.3 mm. coleoptile sections.

4. There are great differences in transport velocity among various growth promoting substances. Owing to this and to differences in the rate of the growth reaction and limited transport capacity of the coleoptile cells, a wide variation results in the apparent growth activity of these substances in the *Avena* test.

5. Transport of indoleacetic acid through other plant tissues was determined, with positive results in corn coleoptile, papaya leaf stalks and midribs, *Tropaeolum* leaf stalks and flower stalks, and *Nitella* internodal cells.

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CYTOLOGY OF DORMANCY IN PHASEOLUS AND ZEA

FREDERIC G. MIDDENDORF

(WITH NINETEEN FIGURES)

Introduction

Dormancy of plant seeds and spores, as well as the analogous problems in animal life of encystment, desiccation, aestivation, and hibernation, have been a common source of research in the recent past. Excluding the studies on seed coats, however, little cytological work has been done on the dormant seed. The cytology of dormancy in *Zea* has been studied by HICKERNELL (**13, 14**) and in *Phaseolus* by KATER (**16**). Both investigators described a migration of chromatin as the important phenomenon. HICKERNELL portrayed a migration of chromatin from the periphery of the dormant nucleus to the center in the germinated or active nucleus of corn and the rotifer; KATER described just the opposite migration in the bean, concluding that the dispersed chromatin in the predormant nucleus migrated via the linin threads into the nucleolus of the dormant cell and returned to the normal dispersal at germination. MACDOUGAL, LONG, and BROWN (**18**) investigated the cytology of active and desiccated *Echinocactus*, and agreed with HICKERNELL in describing a migration of chromatin from the periphery of the desiccated nucleus to the center of the active one. They state that during recovery, "the granular material that had accumulated in the peripheral portion of the nucleoplasm migrates to the central region of the nucleus" (p. 325).

The initial aims of the present investigation were: (1) to check the previously described cytological basis of dormancy and to determine which of these migrations is correct, or whether there is a migration at all; (2) to establish further, if possible, a more general morphological basis of dormancy.

Material and methods

Phaseolus lunatus var. *lunonanus* (dwarf or baby lima bean), *Zea mays* var. *indentata* (dent corn), and *Pisum sativum* (garden pea)

were the types chosen for observation. As a rule only the hypocotyl of both dormant and germinated seeds were fixed, for the study was concentrated on pith, cortex, and vascular areas of the radicle. For germination, seeds were soaked in warm tap water for several hours until the seed coats were completely expanded, then placed on moist filter paper in petri dishes for about two days at room temperature. Growth was faster and roots were thicker if the seeds were kept on moist filter paper in a germinator at 30° C.

A number of fixations were chosen as likely to give varied results: (1) Karpechenko's chrome-acetic-formalin; (2) Flemming's strong solution, chrome-acetic-osmic; (3) sublimate-acetic-alcohol; (4) Regaud's formol-bichromate; (5) Mann-Kopsch's sublimate-osmic. Sections were cut usually 4 μ thick.

Two stains were employed after each of the fixations except the last: Heidenhain's iron haematoxylin and basic fuchsin for the Feulgen reaction. For the iron haematoxylin stain following the Karpechenko, Flemming, and sublimate fixations, sections were mordanted in 2 per cent iron-alum for five minutes at 40° C., stained in 1 per cent haematoxylin at 40° C. for ten minutes, then destained in 2 per cent iron-alum. Following Regaud's fixation, sections were mordanted in 3 per cent iron-alum for one hour at 37° C., stained for two days in 2 per cent haematoxylin at room temperature, then destained in 3 per cent iron-alum. For the Feulgen reaction, hydrolysis was effected by treating the sections of material fixed in Karpechenko's, Flemming's, or Regaud's with normal HCl at 50° C. for twenty to twenty-five minutes. A five minute hydrolysis was used for sublimate preparations. Hydrolysis was preceded and followed by a two minute treatment in normal HCl at room temperature. Sections were then stained in fuchsin-sulphurous acid for at least three hours and washed in three or more changes of SO_2 water.

Drawings were made with the aid of a camera lucida from a Leitz fluorite objective and a no. 15 compensating eyepiece. With one exception, cells to be illustrated were chosen just above the area of division or what corresponds to that area in the dormant radicle. In fact, attention has been concentrated on this locality during the entire study, because of the obvious activity of these cells during germination.

The following method was employed to determine water content of the dormant radicle. The radicles of a great number of seeds were dissected out, weighed, desiccated in an oven at 70° C. for a week, then reweighed and the percentage of water calculated. The percentage of water of radicles clipped from germinated seeds was determined in the same manner.

Germinated seeds were artifically dried for cytological study in a desiccator overnight at 37° C.

Observations

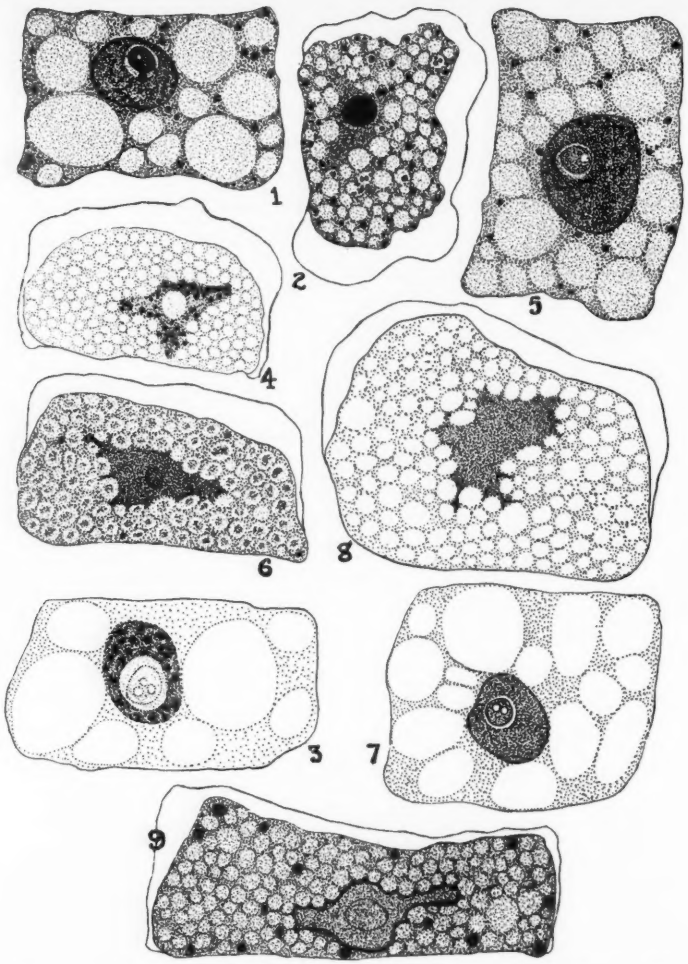
Plasmolysis

Plasmolysis is the most obvious characteristic of the dormant cell. It is strikingly evident in the cotyledon, radicle, and plumule of dormant bean and pea, and in the cotyledon, endosperm, and radicle of dormant corn, but it is almost absent from the plumule of the latter. This is due no doubt to the arrangement of cells in layers two or three rows thick. Possibly when water is lost the whole leaf primordium shrinks instead of the individual protoplast shrinking from the cell wall. The cells of the dormant radicle of the bean, pea, and corn appear characteristically to plasmolyze in pairs facing each other (fig. 19). Artificial desiccation of germinated radicles did not produce plasmolysis. The cell walls shrink with the protoplast, usually distorting or rupturing both cell wall and vacuoles.

Nucleus

Germinated seeds.—The germinated nucleus is turgid and plump; its shape is normally spherical, with a few oval or lobose amoeboid forms (figs. 1, 3, 5, 7, etc.). It is bounded by a definite limiting membrane which seems to be darkly stained by iron haematoxylin (figs. 1, 5). This membrane does not react to the Feulgen test, but is differentiated by prior fixation.

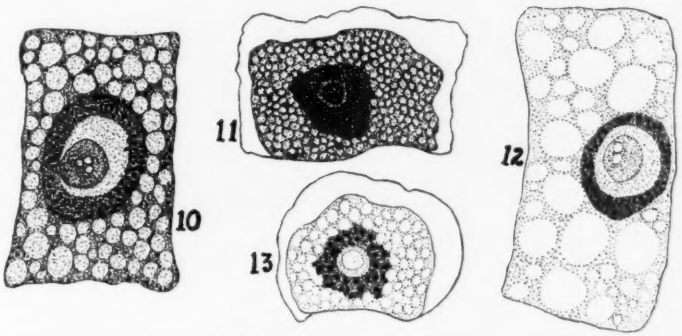
A coarsely granular reticulum is demonstrated by the Feulgen reaction following Karpechenko's (figs. 3, 12), sublimate, and Regaud's fixations. Flemming's fixation gives a finer reticulum (fig. 7). The reticulum of corn stains more brilliantly than that of the bean when both are given the same treatment. This suggests a greater concentration of nucleic acid in corn. Similarly it may be



FIGS. 1–9.—Drawn from cortex of bean radicle with camera lucida, fluorite oil immersion, and 15× compensating ocular. All from 4 μ sections; ×1966. Fig. 1, germinated, Karpechenko-haematoxylin. Nucleolus contains a small vacuole. Note plastin granule in perinucleolar area, prominent nuclear membrane, and large vacuoles of cytoplasm. Fig. 2, dormant, Karpechenko-haematoxylin, showing solid black nucleolus with slight perinucleolar area; fine granular reticulum of irregular nucleus does not stain much more intensely than the cytoplasm. Note absence of nuclear membrane and small vacuoles of cytoplasm. Fig. 3, germinated, Karpechenko-Feulgen. Note coarse granular reticulum of oval nucleus. Nucleolus not stained, but differentiated by fixation. Fig. 4, dormant, Karpechenko-Feulgen. Note coarse granular reticulum of stellate nucleus and hyaline center representing nucleolus. Fig. 5, germinated, Flemming-haematoxylin, similar to fig. 1, but nucleus less coarsely granular. Fig. 6, dormant, Flemming-haematoxylin, similar to fig. 2. Nucleolus stained as rest of nucleus; latter readily distinguished from cytoplasm but noticeably lacks a membrane. Fig. 7, germinated, Flemming-Feulgen, showing finer reticulum and smaller perinucleolar zone than fig. 3. Nucleolus stained. Fig. 8, dormant, Flemming-Feulgen, showing finer reticulum than fig. 4. No nucleolus present; other cells normally possessed unstained nucleoli. Fig. 9, dormant, Mann-Kopsch, showing thick structure bounding nucleus after osmication. This membrane appeared hyaline but is represented in black.

concluded that nucleic acid is more concentrated in chromosomes than in the reticulum, for the former stain more intensely than the reticulum of the resting nucleus.

The nucleolus is always evident in germinated seeds, even if not stained. Iron haematoxylin stains it black, usually with a more dense rim or border and a lighter center (fig. 1). This center is normally vacuolated (fig. 10), and the black rim frequently has one or



FIGS. 10–13.—Corn radicle, Karpechenko fixation. Drawn from cortical parenchyma with camera lucida, fluorite oil immersion, and 15× compensating ocular; 4 μ sections; ×1966. Fig. 10, germinated, haematoxylin, similar to fig. 1 except for larger perinucleolar zone and smaller vacuoles in cytoplasm. Fig. 11, dormant, haematoxylin, similar to fig. 2 except that nucleus is less irregular and nucleolus does not stain darker than rest of nucleus. Fig. 12, germinated, Feulgen, similar to fig. 3. Fig. 13, dormant, Feulgen, similar to fig. 4.

two jet black spherical granules adjoining it in the perinucleolar area (fig. 1). CARLSON (7) considers this granule of a plastin nature. The Feulgen reaction normally leaves the nucleolus unstained. Some Flemming material, however, shows nucleoli stained (fig. 7); and after Karpechenko's a few nucleoli appear lightly colored, especially around their border. This was also observed by CARLSON following certain fixatives. The preceding fixations differentiate a boundary and an internal vacuolate structure which are just as evident when the nucleolus remains uncolored as when some of the stain is deposited (figs. 3, 12).

The perinucleolar area, usually very evident in germinated seeds, is normally larger in corn than in bean; compare figures 1 and 3 with figures 10 and 12.

DORMANT SEEDS.—Dormant nuclei lack the plumpness of germinated ones; instead they were shrunken to a stellate or crenate form. A comparison of figures 3 and 4 with figures 12 and 13 illustrates that departure from the normal, spherical, nuclear type of the active plant is much more noticeable in the bean than in corn. In the latter, pith, cortex, and vascular areas contain crenated nuclei (fig. 13), among which vascular nuclei are the smallest. In the bean, pith and cortical nuclei are irregularly stellate or of almost any possible shape, the processes wedging between the vacuoles of the cytoplasm (figs. 6, 8). Vascular nuclei are merely crenated as in corn.

No stained or even differentiated limiting structure or membrane can be observed in the dormant nucleus following Karpechenko's (figs. 2, 4, 11, 13), Bouin's, Regaud's, or sublimate fixations. Usually after Mann-Kopsch's fixation a membrane is strikingly evident and exaggerated (fig. 9). Such a membrane also appears occasionally after Flemming's strong, and is probably due to osmication. After the first named fixations, haematoxylin stains the nucleus gray or black, the periphery of which, especially in the bean when sufficiently destained, is scarcely distinguishable from the cytoplasm (fig. 2). The color abruptly fades out or blends with that of the cytoplasm between the vacuoles. The Feulgen reaction likewise shows no structure limiting the dormant nucleus, nor is one differentiated by prior fixation. The invisibility of this supposed membrane after the majority of fixations is significant and indicates its probable absence in the dormant cell. Possibly some conclusion regarding the nature of the nuclear membrane can be drawn from the phenomenon. If the structure disappears upon the loss of water, as it apparently does, it is scarcely a true morphological membrane as stated by HICKERNELL (**14**) for corn and rotifer, but is more plausibly regarded simply as an interface.

The Feulgen reaction stains a reticulum after the majority of fixations as a cluster of purple-red granules or flakes (figs. 4, 13). After the Flemming fixation the reaction often gives a more even deposition of color (fig. 8). The fact that the reticulum of the cells of the dormant radicle is more intense in its reaction to the Feulgen technique than that of cells of the germinated radicle suggests that chromatin is more concentrated or more compact in the dormant seed.

Although the nucleolus is an outstanding structure of the dormant nucleus stained by haematoxylin, the Feulgen reaction leaves it unstained; compare figures 1 and 4, or figures 11 and 13. In the bean, haematoxylin stains the nucleolus solid black (fig. 2); it is sometimes slightly irregular in outline (fig. 15), but has no internal vacuolate structure as was observed in this organelle in the germinated seed. In dormant corn, the nucleolus may stain more lightly than the reticulum, as described by HICKERNELL (**13, 14**), but only after a short haematoxylin stain. In this case, too, no internal vacuolate structure is evident (fig. 11). Not only does the Feulgen reaction fail to stain nucleoli, but the preceding fixation also leaves them undifferentiated. However, a spherical, hyaline, central area may be interpreted as representing one (figs. 4, 13).

The perinucleolar area is slight or absent in dormant cells.

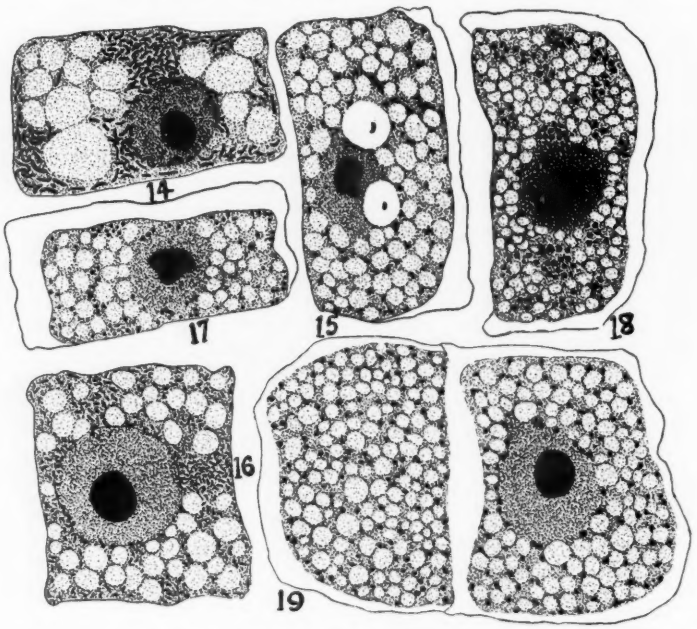
CYTOPLASM

GERMINATED SEEDS.—Vacuoles of the germinated cell vary in size, depending on the position of the cell in the radicle. Cells nearest the root tip are packed with small vacuoles. As the cell becomes farther removed from the root tip the vacuoles gradually enlarge. Cortex and pith of the same region contain vacuoles of similar size which are slightly larger than those of the small vascular cells.

Although it seems advisable to distinguish with SOROKIN (**22**) between "mitochondria" and "primordia of plastids," if this is possible, such a distinction has not been made in the present work, since the Janus green B test has not yet been applied. Consequently the term mitochondria is used to include both "mitochondria" and "plastid primordia."

In the germinated seed, mitochondria are crowded between the vacuoles (figs. 14, 16). They may be rather evenly distributed throughout the cell or concentrated around the nucleus. One bean radicle possessed solely long filaments, spiral or horseshoe-shaped or with ends attached to form a ring. But the normal mitochondrial complex of germinated seeds consists of rods with some filaments, granules, and hollow spheres. This agrees with descriptions of N. H. COWDRY (**9**) for the pea, and ANDERSON (**1**) for the hyacinth. Bean mitochondria are somewhat larger than those of corn as fixed by Regaud's method; compare figures 14 and 16.

DORMANT SEEDS.—Vacuoles of the dormant seed vary slightly in size within a single cell, but are universally smaller and more numerous than those of germinated seeds. Perhaps this could be interpreted as indicating that a multiplication of vacuoles is associated with dormancy. The tonoblasts or boundaries of the vacuoles remain just as evident in the dormant cell as in the germinated one. Further, dormant vacuoles do not lose their spherical or oval shape as does the nucleus.



FIGS. 14–19.—Drawn with camera lucida, fluorite oil immersion, and compensating ocular 15×. All from 4 μ sections; ×2200; Regaud's method for mitochondria. Some mitochondria drawn above the nucleus. With exception of fig. 18, all taken from cortex just above dividing area (or its equivalent in dormant seed). Fig. 14, germinated bean showing mitochondria as rods. Fig. 15, dormant bean showing mitochondria as granules. Two starch grains evident with hyla stained black. Fig. 16, germinated corn showing mitochondria as short thin rods. Fig. 17, dormant corn showing granular mitochondria. Fig. 18, dormant pea, taken from tip of hypocotyl and showing granular mitochondria in groups. Fig. 19, taken from cortex of same embryo as fig. 18 but showing granular mitochondria uniformly distributed in cytoplasm. Note how cells plasmolyze in pairs.

I had intended to confine this study to a comparison of the bean and corn, but results obtained appeared to contradict a statement of E. V. COWDRY (**8**) regarding the mitochondria of the dormant pea. He states: "it has been found by N. H. COWDRY that they [mitochondria] are filamentous in the rather inactive cells of the dried seed pea" (p. 315). Sections of the dormant garden pea were then made, and the mitochondrial type of the pea was found to agree perfectly with that of the dormant bean and corn.

In the dormant cell mitochondria again are crowded between the vacuoles (figs. 15, 17, 19). As for germinated seeds, so for dormant seeds, mitochondria may be evenly scattered throughout the cell or

TABLE 1

CYTOLOGICAL DIFFERENCES BETWEEN DORMANT AND GERMINATED SEEDS

	DORMANT SEEDS	GERMINATED SEEDS
Plasmolysis	Present	Absent
Shape of nucleus	Shrunken, irregular, stellate	Turgid, regular, spherical
Nuclear membrane	Not visible	Visible
Nucleolus	Not differentiated	Differentiated
Perinucleolar area	Small or none	Large
Vacuoles	Small	Large
Mitochondria	Small spheres	Rods, spheres, and filaments

concentrated in groups around or away from the nucleus. Usually the cells in one area all possess a similar distribution of mitochondria. Such a region may embrace pith, cortex, and vascular areas. Figure 18 shows grouped mitochondria in a cortical cell near the root tip of the dormant pea. Cells some distance above this point in the same hypocotyl possessed an even distribution of mitochondria (fig. 19). Dormant mitochondria are all of the small granular type, with a very few short shrunken rods. With Regaud's method the granules are usually not perfect spheres but pebble-shaped or shrunken. Pea and bean are almost identical in size, shape, and number of mitochondria—about one hundred per cell per 4 μ section. Mitochondria of corn are very similar but somewhat smaller.

Two large starch grains appear in figure 15. If the hylum is present in the section, it is stained prominently by haematoxylin as

shown in the drawing. In the present work, however, the distribution of starch in bean and corn was not studied. Its distribution in the hypocotyl and plumule of the predormant, dormant, and germinated bean has been described by KATER (16).

The characteristic cytological differences between the germinated and dormant seeds thus far described have been summarized in table 1.

The water weight of the bean and corn were determined and found to be 6.015 per cent of the dormant bean hypocotyl, 2.22 per cent of the dormant corn hypocotyl, 91.86 per cent of the germinated bean radicle, and 89.31 per cent of the germinated corn radicle. It will be noticed that the bean possesses a larger percentage of water than corn in both the dormant and germinated states, but that during germination the corn acquires a larger percentage of water (87.09 per cent) than does the bean (85.85 per cent).

Discussion

Since its publication by FEULGEN and ROSSENBECK (12), the nucleal reaction has been applied to almost every type of cytological material. Literature in regard to the Feulgen reaction is considerable and contradictions not uncommon. For instance, FEULGEN and ROSSENBECK (12) first claimed that yeast gives absolutely no reaction; subsequent observations of MARGOLENA (19), PETTER (20), ROCHLIN (21), and others have shown that this important biological group may give a very intense reaction. A positive reaction for Nissl bodies was reported and denied (for literature see 4 and 23). Further, statements regarding the reaction given by eggs in various stages do not agree. FEULGEN (11) reported unripe eggs and fertilized ripe ones to give a positive reaction, unfertilized ripe eggs to give no reaction. BAUER (2) states that developing eggs of most animals give no reaction. To mention but two recent American workers, BLUNT (5) reports a positive reaction given by nucleoli (net-knots) of early oocytes in *Cambarus*, whereas JOHNSON (15) reports a negative reaction for the entire nucleus of crayfish eggs during the growth stage. It has become a commonplace to pay tribute to the specificity of the reaction for chromatin; but incongruous statements

such as those referred to indicate that an accurate interpretation of the Feulgen reaction demands experience and checking.

The type of fixation modifies the reaction somewhat; one fixation demands a longer or shorter time of hydrolysis (**2**) and shows a coarser or finer reticulum (**23**) than another. Hydrolysis weakens the absolute validity of morphological conclusions based on the reaction, for, besides distorting the tissue, it may dissolve the nucleic acid and deposit it in a nearby area of the cell, as observed by BERG (**4**) and CARLSON (**7**). The reaction in these instances would not be locally specific. Further, cell elements or products, such as starch and cellulose, may stain with the reaction (**3, 7, 19**).

In this work several fixations were used as checks on one another. Two of these, chrome-osmic and chrome-formalin, are reported by BAUER (**2**), who tested for the optimum time of hydrolysis following twenty-four different fixations, as completely resisting distortion by hydrolysis. Study of a great number of slides of material prepared from different fixations indicated that only occasionally did color appear in or on the border of the nucleolus. With the following exceptions, no extra-nuclear structures were observed stained by the reaction: (a) Cell walls of xylem elements of germinated seeds were occasionally stained intensely after any fixation; cell walls of non-vascular tissues of both germinated and dormant seeds rarely stained except after chrome-osmic. (b) Starch grains stained deeply after chrome-osmic, and in one set of slides after sublimate-alcohol fixation. No starch grains were observed stained after chrome-formalin and formol-bichromate. The staining of these structures, however, scarcely offers occasion for confusion with nuclear material, by reason of their form and position. They also give the reaction after a period of hydrolysis insufficient to produce color in the nucleus. In fact, according to MARGOLENA (**19**), they react without any prior hydrolysis.

Once the loopholes in the validity of the reaction are checked, the Feulgen reaction can be relied on as a specific microchemical test for nucleic acid indicating both the presence and the morphology of chromatin in the cell.

Previous investigators studying the cytology of dormancy have stressed a migration of chromatin as the basic morphological con-

comitant of latency. Although agreeing on this general point, interpretations have differed greatly in specific detail, HICKERNELL (**13, 14**) believing the movement to be centrifugal; KATER (**16**) regarding it as centripetal. This disagreement alone might cast suspicion upon the validity of the techniques employed; and after the present study of Feulgen material it is possible to reinterpret the role played by chromatin in dormancy of seeds. In brief, these opposite descriptions of chromatin migration must be discarded in their entirety.

In the first place, the reaction shows no striking difference in the distribution of chromatin of the dormant and germinated seeds. The fact that the stain is somewhat more intense in the dormant than in the germinated nucleus may indicate that chromatin, which we identify with nucleic acid, is more concentrated or more compact in the dormant seed. Probably, however, there is not a greater amount of nucleic acid in the entire dormant seed. It would seem rather that the same amount is crowded into a smaller space in the dormant nucleus because of shrinkage. But apart from this, chromatin or the reticulum scarcely offers a source of cytological difference between dormant and germinated seeds.

In the second place, dormant and germinated nucleoli do not appear to differ in kind; both react negatively to the Feulgen technique and seem to be of the nature of a plasmosome. Others have repeatedly observed that the Feulgen reaction does not stain plant nucleoli (**2, 4, 7, 19, 24,** *et al.*). In the present work, dormant nucleoli never stained. In the germinated seeds they occasionally stained or showed a light color around the border, but only after the use of a few fixatives. CARLSON made a similar observation and decided that chromatin dissolved by hydrolysis accumulated there. This conclusion seems more sound than any other that suggests itself. Chromatin of the dormant nucleus is probably less soluble, and hence does not accumulate in or near the nucleolus. We may reasonably state that chromatin is not present in either the dormant or the germinated plant nucleolus. All possibility, therefore, of a migration of chromatin from the periphery to the nucleolus or vice versa is excluded. Even though we recognize the possibility that an aldehyde group may be introduced by fixation into a constituent of the nucleolus and so allow for the positive reaction of some nucleoli, it

still would scarcely indicate the presence of nucleic acid, but rather a reaction similar to that of some fixations and starch.

KATER's interpretation grew out of his error in regarding the nucleolus and perinucleolar zone as constituting the entire dormant nucleus and in disregarding the very lightly staining chromatin outside the perinucleolar zone. This mistake is easily made in well destained slides since the dormant nucleus lacks a visible membrane and the boundary between nucleus and cytoplasm is often determined with difficulty, especially after the Bouin or Karpechenko fixations. The significance of HICKERNELL's descriptions I cannot state, but it is certain that there is not a migration of chromatin in the dormant bean and corn, or the Feulgen reaction would show it.

All the described cytological differences between the dormant and germinated seed would seem to be correlated with water content. Obviously plasmolysis and perhaps the shrunken irregular shape of the dormant nucleus and the concomitant loss of a visible nuclear membrane and other internal nuclear changes result from loss of water. Recently KATER (17) correlated the size and shape of mitochondria with the water content of the liver in the rabbit. Increased percentage of water in the liver caused enlargement and enspherulation of mitochondria. In the plant the great increase of water content in the germinated seed is accompanied by enlargement of mitochondria, but the characteristic morphological types are rods and filaments rather than spheres. The fact that both bean and corn show the variation from granules in the dormant to enlarged rods in the germinated seed suggests constancy for the phenomenon in the plant. Water probably influences the size of mitochondria directly but their shape only indirectly.

Summary

1. The cellular morphology of the dormant and the germinated seed was compared in both the bean and corn. Seeds were fixed in various fluids, and after each type of fixation were stained by both iron haematoxylin and the Feulgen reaction.

2. In the dormant state the cell is plasmolyzed; the nucleus has an irregular form, apparently lacking a membrane or limiting structure; the nucleolus is not optically differentiated by fixation although

it may stain intensely with iron haematoxylin. The vacuoles are small, and the mitochondria are small granules or spheres.

3. At germination the cell becomes turgid; the nucleus regains a normal spherical form and the boundary becomes definite; the nucleolus becomes optically differentiated even when unstained as by the Feulgen reaction, and shows a vacuolate structure. The vacuoles of the cytoplasm enlarge, and the mitochondria elongate into rods or filaments.

4. Prior concepts regarding the chromatin of the dormant cell as having migrated centrifugally (HICKERNELL) or centripetally (KATER) are rejected on the basis of the Feulgen reaction.

I am indebted to Dr. J. McA. KATER for constructive criticism throughout the work as well as for the use of his slides of seeds fixed in Bouin's and stained with iron haematoxylin. Thanks are also due to Dr. R. A. MUTTKOWSKI for assistance in the preparation of the manuscript.

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EFFECTS OF INDOLEACETIC AND NAPHTHYLACETIC ACIDS ON DEVELOPMENT OF BUDS AND ROOTS IN HORSERADISH[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 497

ROBERT C. LINDNER

(WITH FOURTEEN FIGURES)

Introduction

In recent years a number of investigators have emphasized the influence of auxins and similar compounds on the growth processes of plants (**4, 25**). It was observed that the application of relatively high concentrations of these substances stimulates the production of numerous adventitious roots from stems and other above-ground portions of various plants (**1, 3, 6, 7, 9, 10, 11, 13, 15, 22**). The stimulation in lateral root production from aerial roots has also been reported (**19**). It then became of interest to determine the response of a fleshy root to applications of various auxins at relatively high concentrations. The fleshy root of horseradish, *Cochlearia armoracia*, was chosen for this investigation because it produces both roots and shoots readily when cut into transverse segments and placed in a favorable environment. This plant also permits the study of the effects of certain growth influencing substances on the production of buds as well as roots.

Material and methods

The horseradish roots were obtained from a market in Chicago during November, 1937, and stored in a cool basement until used. In most of the work the roots were cut into cylindrical segments about 4 cm. long, and a small notch was made in the lower face of each segment to distinguish the morphologically lower end. The extreme upper and lower segments were usually discarded, those from the central portion being used for the experimental purposes.

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

More than 1200 such pieces of root, representing derivatives from more than 300 plants, were used in the course of the following treatments:

1. Saturated aqueous solutions of indoleacetic acid or naphthylacetic acid applied with a brush to the upper, lower, or both cut surfaces.

2. Two per cent mixtures of indoleacetic or naphthylacetic acids in lanolin smeared on upper, lower, or both cut surfaces.

3. Pure lanolin smeared on upper, lower, or both cut surfaces.

4. Two per cent mixtures of indoleacetic or naphthylacetic acid in lanolin applied as a band (about 5 mm. wide) around the peripheral face of the segments near the upper cut surfaces.

5. Untreated.

The segments treated with pure lanolin will be considered the controls, since they responded in a manner similar to the untreated ones. The sections were placed in moist chambers, moist sand, or moist sphagnum moss, and about 2 weeks later the amount of root and bud formation was observed. All experiments were duplicated with root segments placed in running tap water for 24 hours before treatment.

Some roots were split longitudinally down the middle, and to one cut surface was applied pure lanolin, and to the other 2 per cent mixtures of either indoleacetic or naphthylacetic acid in lanolin.

For histological study, material was obtained from segments whose upper cut surfaces had been treated with 2 per cent naphthylacetic acid in lanolin. The response to indoleacetic acid was essentially the same as that for naphthylacetic acid, except that it was less intense, and will therefore not be described. Segments similarly treated with pure lanolin were used as controls. Daily samples were taken. The material was fixed in Navashin's solution, and run through an n-butyl alcohol series into paraffin. Sections were cut at 8, 10, and 12 μ.

Gross observations

The segments which were untreated or treated with pure lanolin produced roots and shoots from the lateral surfaces in the region of the lateral root traces. Shoots were produced near the morphological top of the segment and roots near the morphological bottom,

regardless of the orientation of a segment during the regeneration period. Buds were formed from the morphologically upper cut surface only in association with a lateral root trace. Roots were occasionally formed from the lower cut surface that were not in association with a root trace.

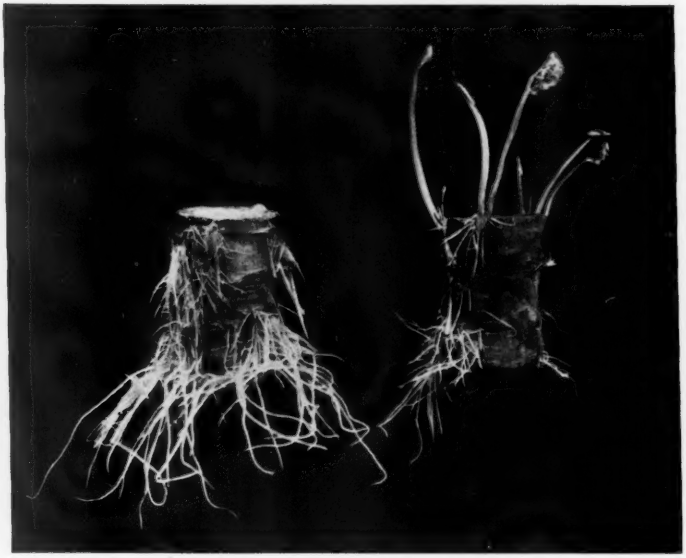

FIG. 1.—Segments of horseradish root, about 4 cm. long, 3 weeks after treatment. Upper surface on left was smeared with 2% naphthylacetic acid in lanolin, while the other segment was treated with pure lanolin. Maintained in moist chamber at about 25° C.

In all cases the application of growth substances induced a striking increase in the quantity of roots and a decrease in the number of buds, and in some instances complete inhibition of the latter (fig. 1). In addition to the numerous roots from the lateral surfaces, the treated segments produced a number of roots at the morphologically lower cut surface, most of them from the cambial region and the phloem. Another effect was a greater formation of callus from the treated surfaces. In the region of peripheral application, growth substances induced the formation of roots that were not in association

with root traces. Table 1 indicates the results of a typical experiment.

When the extreme lower segment of a root was used, it reacted like the others. On the other hand, the uppermost one always produced more roots and buds than any of the other pieces. This upper portion is composed of the stem and the juncture of the stem and root, while the rest of the segments consist only of root.

TABLE 1

EFFECTS OF 2 PER CENT LANOLIN MIXTURES OF INDOLEACETIC ACID AND NAPHTHYLACETIC ACID ON TRANSVERSE SEGMENTS OF HORSERADISH ROOT

TREATMENT	NO. OF SEGMENTS	TOTAL NO. OF ROOTS	TOTAL NO. OF BUDS	AV. NO. OF ROOTS PER SEGMENT*	AV. NO. BUDS PER SEGMENT*
Lanolin on bottom	24	434	286	18± 4	12±2
Lanolin on top	73	1416	789	20± 4	11±2
Indoleacetic acid on side	52	1220	324	24± 5	6±1
Indoleacetic acid on bottom	53	1290	310	25± 4	6±1
Indoleacetic acid on top	51	2090	109	42±10	2±1
Naphthylacetic acid on bottom	44	2410	43	50±10	1±1
Naphthylacetic acid on side	47	3175	0	70±14	0
Naphthylacetic acid on top	42	3985	0	102±16	0

* Standard deviation of mean = $\sqrt{\frac{\Sigma d^2}{n-1}}$.

Washing the segments for 24 hours in tap water before treatment inhibited the formation of callus and resulted in greater invasion by micro-organisms. Aside from these effects, the segments responded similarly to the unwashed ones.

When the entire fleshy axis of a root was split longitudinally and the cut surfaces treated with pure lanolin, there resulted only a small amount of root and bud development. The majority of roots were produced from the stem portion of the axis, while the buds, although fewer in number, were more or less evenly spaced over the entire axis. Application of the growth substances, however, induced the formation of numerous roots from traces in the stem region, and a few from root traces in the extreme lower portion of the root. The

petioles of bractlike leaves growing from the upper portion of the stem part of the axis responded to the treatment by producing roots at their bases.

Water, ethyl alcohol, acetone, ether, and petroleum ether extracts of leaves, buds, roots, and xylem (of the root) were applied in mixtures with lanolin to the cut surfaces of 4 cm. segments, to determine whether the production of shoots could be stimulated. No positive results were obtained with any of these extracts.

Histological observations

GENERAL ANATOMY OF FLESHY AXIS.—The fleshy portion of the horseradish plant is composed mainly of root, but a small amount of stem and the junction of the root and stem compose the upper part. Development of the root is somewhat similar to that of the ordinary radish, described by HAYWARD (12). The primary tissues of the root develop from three apical histogens. The primary vascular tissues may be diarch to pentarch. Numerous air spaces are found in the cortex, close to the root tip (fig. 2). The pericycle is at first one-layered, but it becomes active just prior to cambium formation and forms a multi-layered region. The outer layer develops a phellogen, the activity of which later results in the rupturing and breaking off of the entire cortex (fig. 3). Radial divisions of the phellogen and its derivatives, as well as derivatives of the pericycle and secondary phloem, keep up with the increase in diameter of the root which results from the activity of the cambium (fig. 4). The mature root is composed primarily of secondary phloem and secondary xylem parenchyma cells filled with starch (fig. 5). The xylem constitutes the bulk of the root, and comparatively large vessels are scattered among the parenchymatous elements. Small strands of phloem, derived from proliferations of secondary xylem parenchyma cells, are scattered throughout the central portion of the xylem. It is difficult to determine whether any pericyclic derivatives remain in an old root, since the peripheral tissues are more or less homogeneous in appearance and are continuous with the secondary phloem. In some old roots there undoubtedly remain some cells derived from the original pericycle. Elongated stone cells are found scattered throughout the outer portions of the phloem, and the

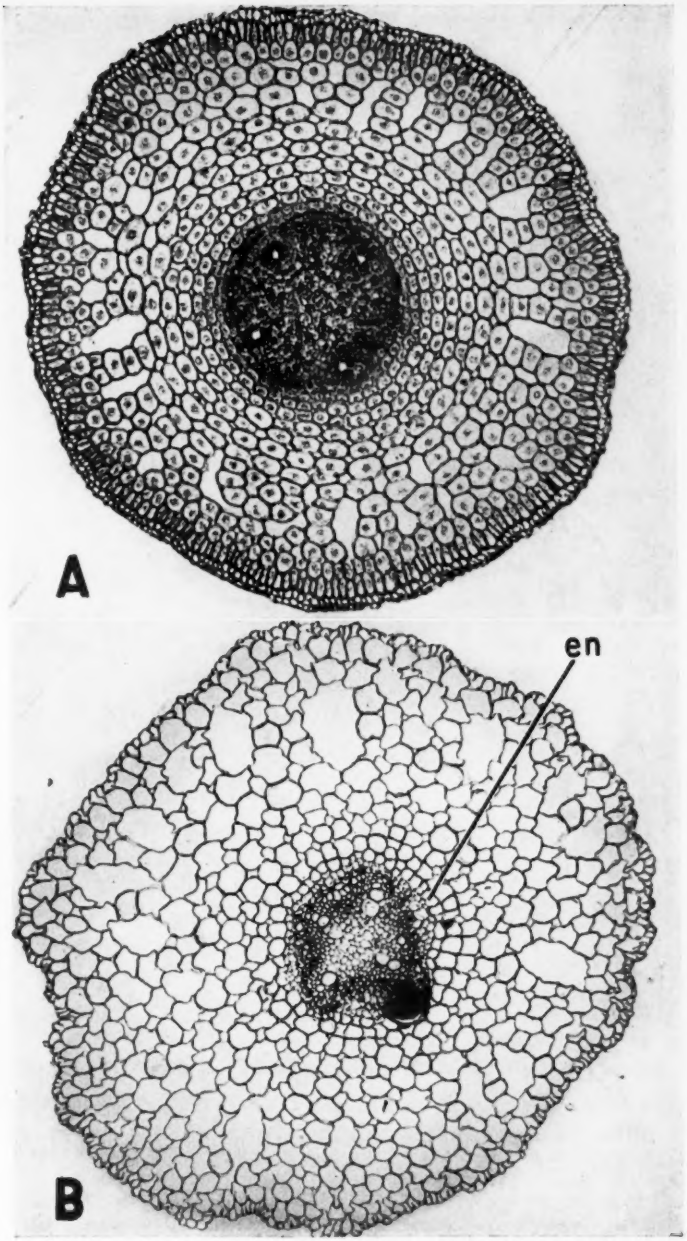

FIG. 2.—*A:* 2 mm. from tip; numerous air spaces in cortex. *B:* 15 mm. from tip; air spaces larger. Lateral root from pericycle. *en*, endodermis.

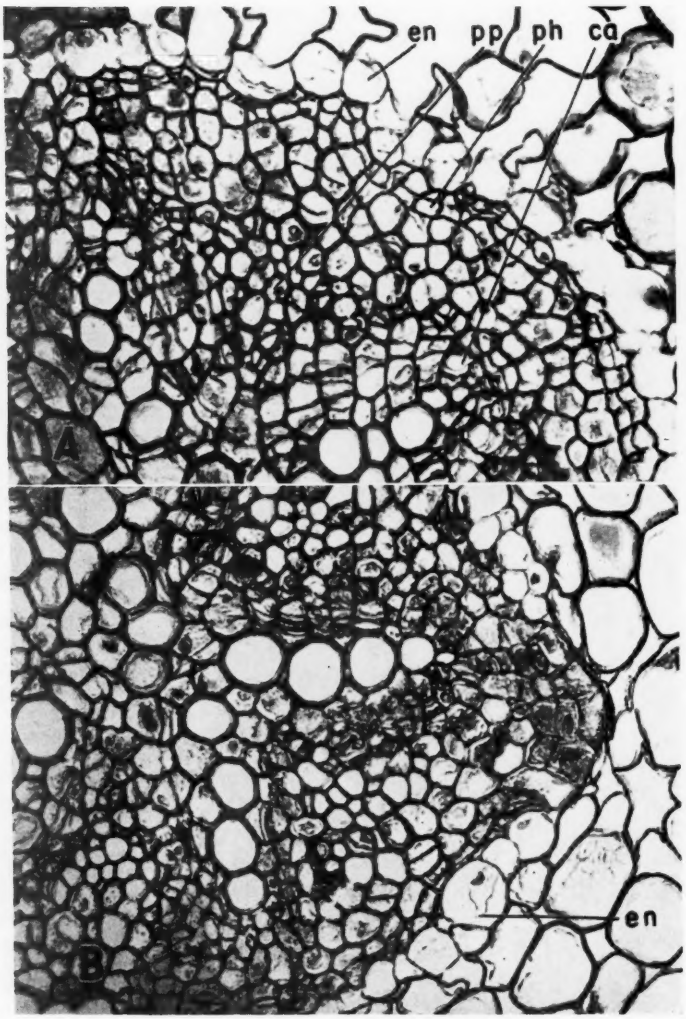

FIG. 3.—Young roots showing beginnings of secondary activity. *A:* parenchymatous derivatives of formerly one-layered pericycle form a band about five cells wide; outer layer of these cells is developing into a phellogen; cambium being formed in parenchymatous cells between primary phloem and xylem. *B:* younger than *A*. Lateral root primordium forming from pericyclic derivatives and crushing endodermis. *en*, endodermis; *ca*, cambium; *ph*, phellogen; *pp*, primary phloem.

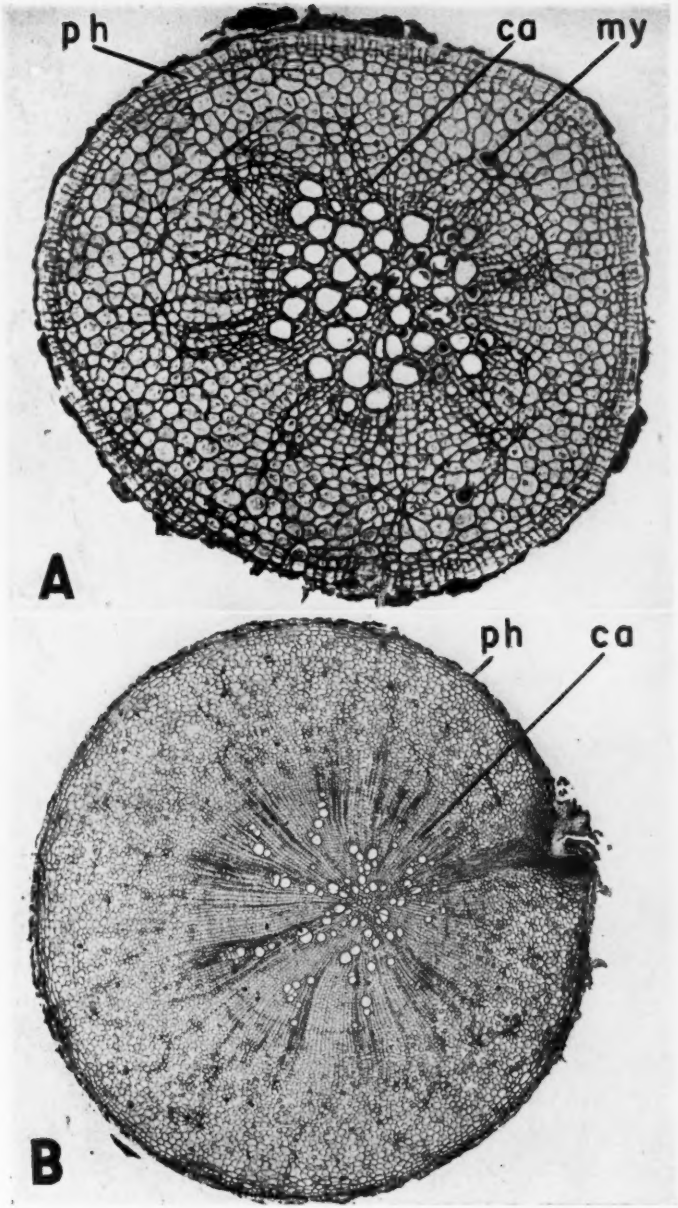

FIG. 4.—Young roots showing secondary activity. *A:* about 1 mm. in diameter. Periderm derived from pericyclic derivatives; cortex absent. *B:* older root, about 3 mm. in diameter, with lateral root trace, and having all structures characteristic of roots 20–30 mm. in diameter, except intraxylary phloem. *ph*, phellogen; *ca*, cambium; *my*, myrosin storage cell.

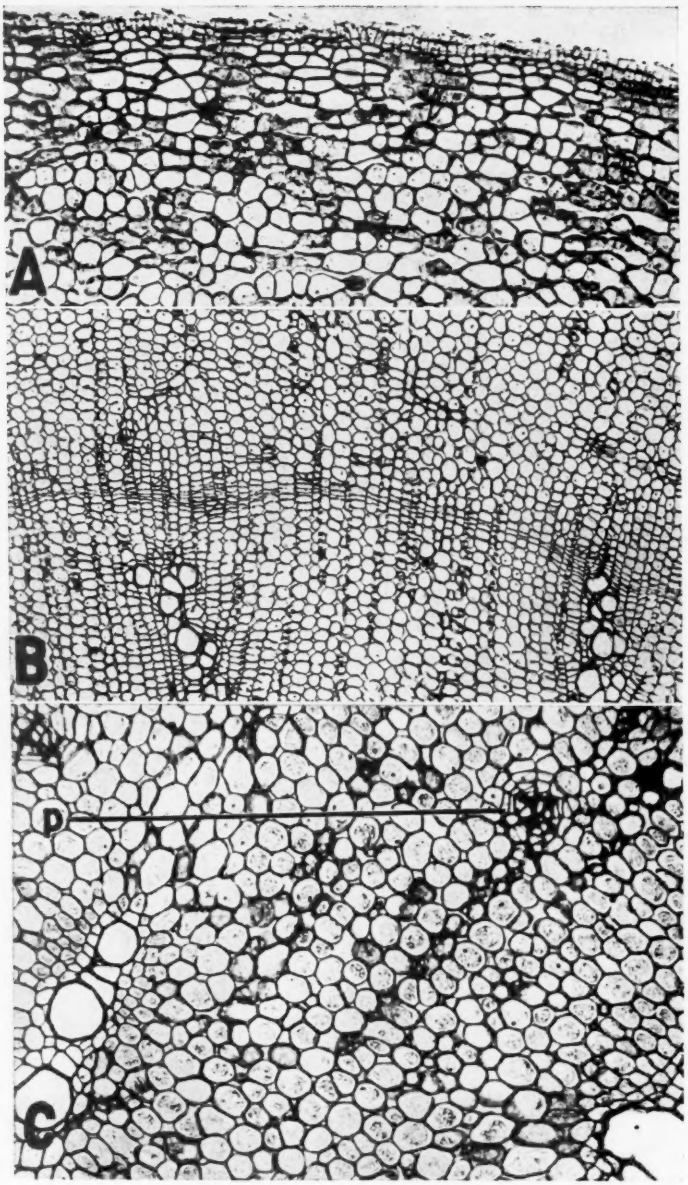

FIG. 5.—Cross sections of portions of roots about 30 mm. in diameter. *A:* periderm and outer phloem. *B:* cambium with associated secondary phloem and xylem. *C:* secondary xylem with strands of intraxylary phloem; parenchymatous cells filled with starch. *p*, phloem strand.

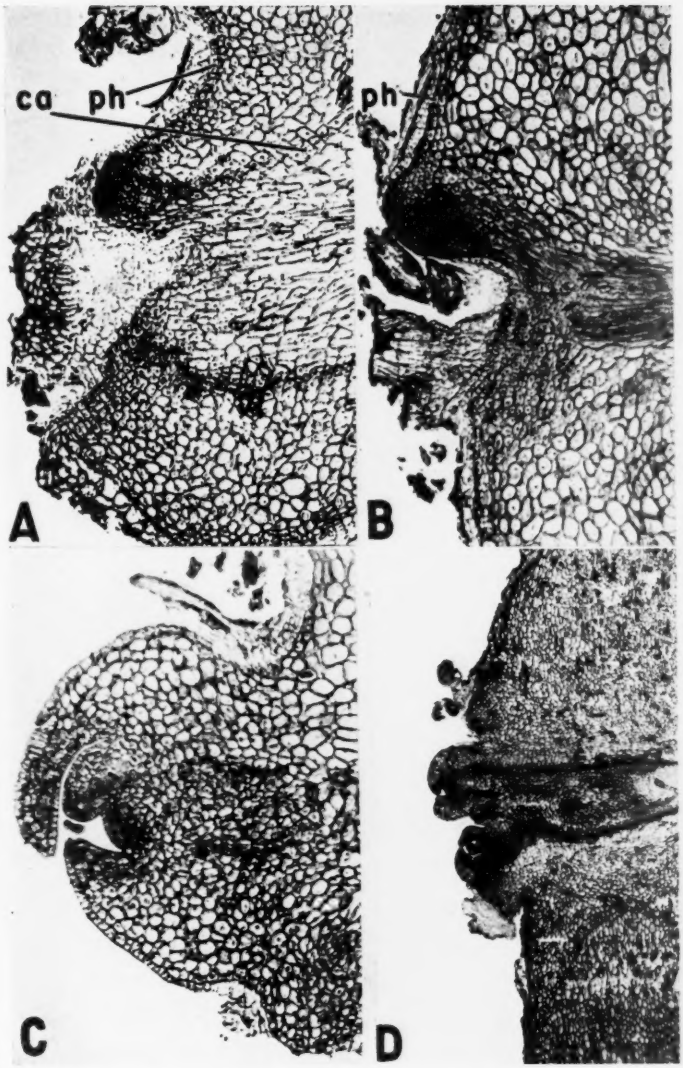

FIG. 6.—Development of buds near lateral root traces of untreated segments. *A:* 4 days old; initial activity involving derivatives of phellogen and cambium of root trace. *B:* 6 days old with one bud developing. *C:* 8 days; developing bud has ruptured periderm; vascular differentiation beginning. *D:* 10 days; vascular tissues of buds connected with those of root trace. *ca*, cambium; *ph*, phellogen.

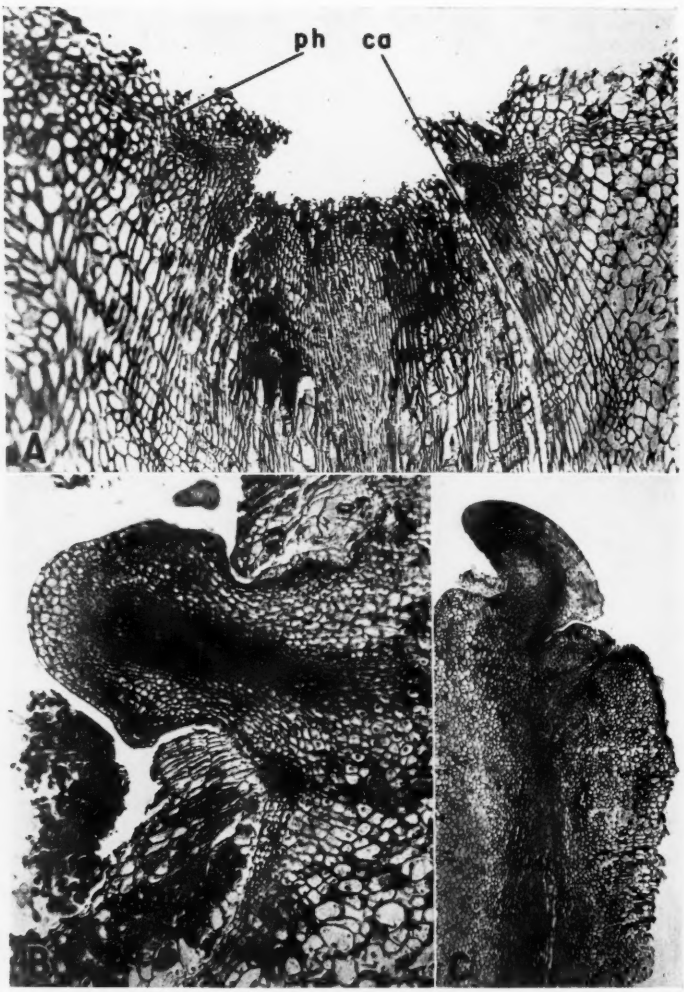

FIG. 7.—Development of roots in treated segments near lateral root traces. *A:* 4 days after treatment; initial stages involving derivatives of phellogen and cambium of lateral root trace (*cf.* fig. 6*A*). *B:* 8 days after treatment. Emergence of young root through periderm (*cf.* fig. 6*C*). *C:* 10 days after treatment, showing well established vascular connections (*cf.* fig. 6*D*). *ca*, cambium; *ph*, phellogen.

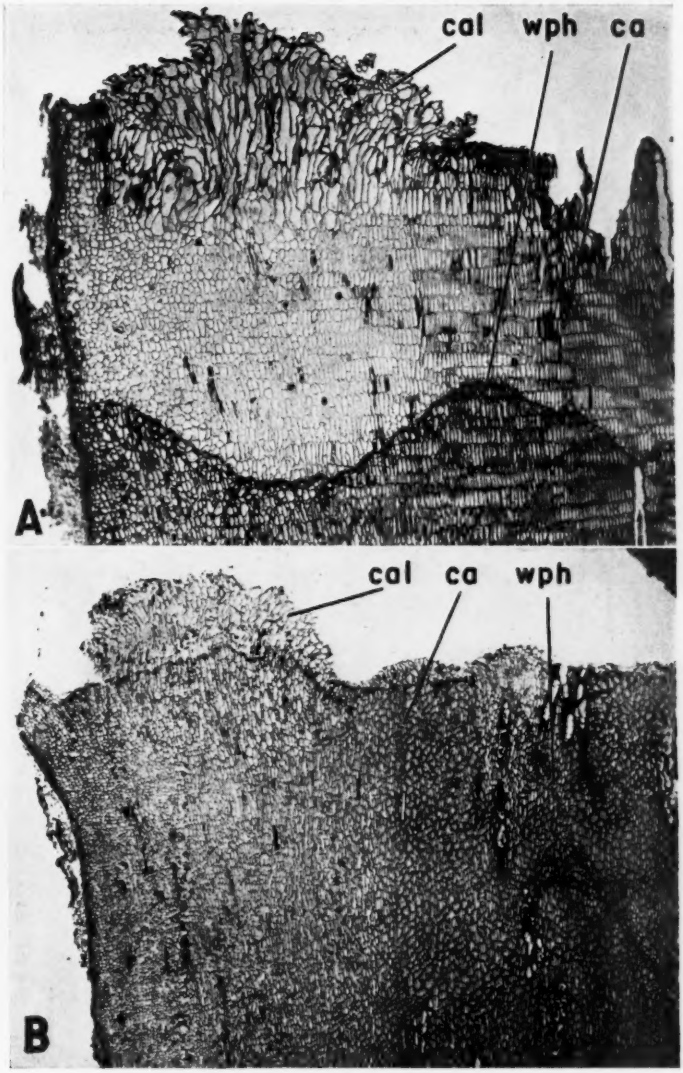

FIG. 8.—Sections through upper cut surface of segments 13 days after treatment. *A:* control. Many cells of phloem and xylem have died; phellogen has formed deep in the phloem and xylem. *B:* treated. Cells have died to considerable depth in xylem; wound phellogen forming; very few dead cells in phloem. *wph,* wound phellogen; *ca,* cambium; *cal,* callus.

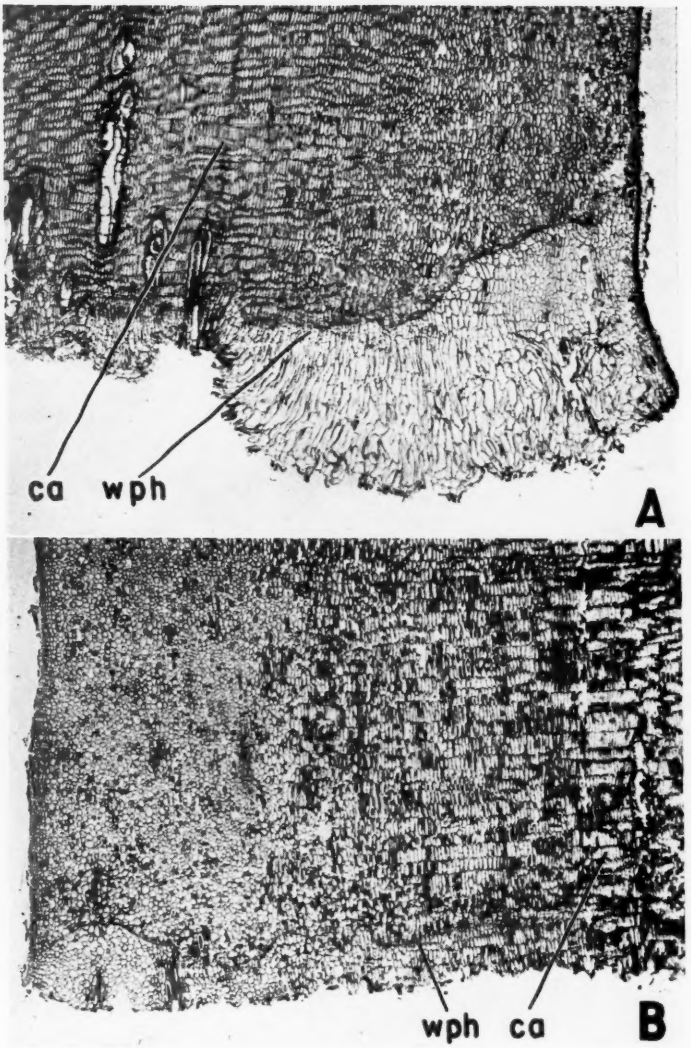

FIG. 9.—Sections through lower cut surface of segments 6 days after treatment. *A:* control. Phellogen is forming that will isolate the calluses. *B:* treated. Very little activity leading to callus formation; phellogen forming. *wph*, wound phellogen; *ca*, cambium.

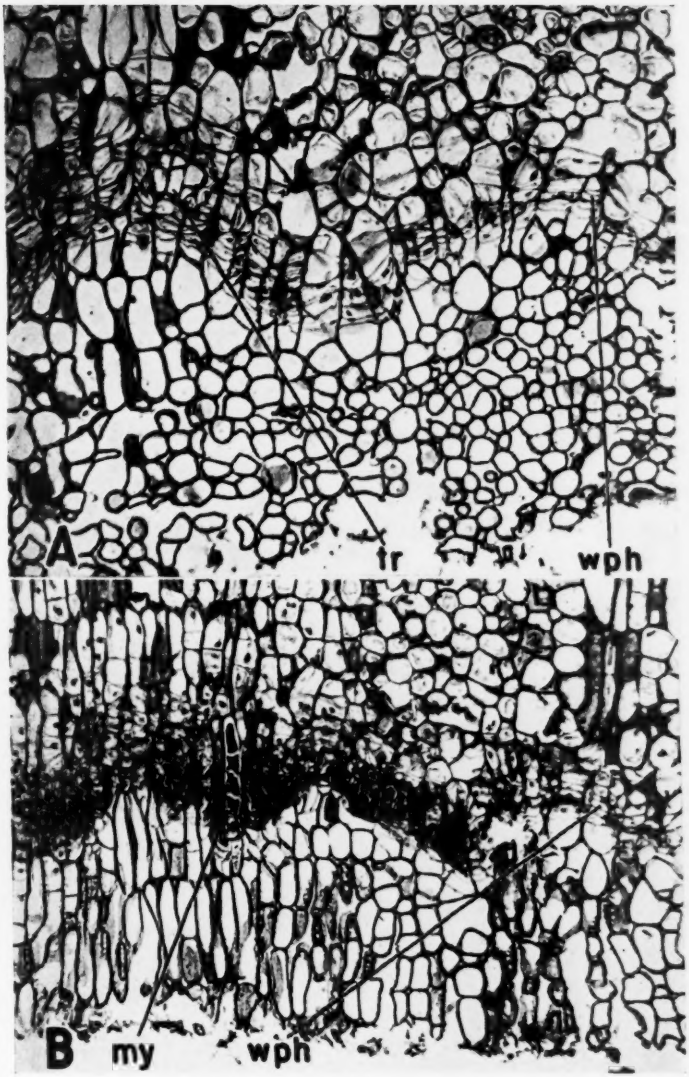

FIG. 10.—Sections through phloem region of lower cut surface of treated segment, 8 days after treatment. *A:* group of isolated tracheids developing from derivatives of secondary phloem parenchyma. *B:* early stage in root formation. Derivatives of wound phellogen and associated secondary phloem parenchyma cells involved. Myrosin storage cell (*my*) has undergone several divisions. *tr*, tracheid; *wph*, wound phellogen.

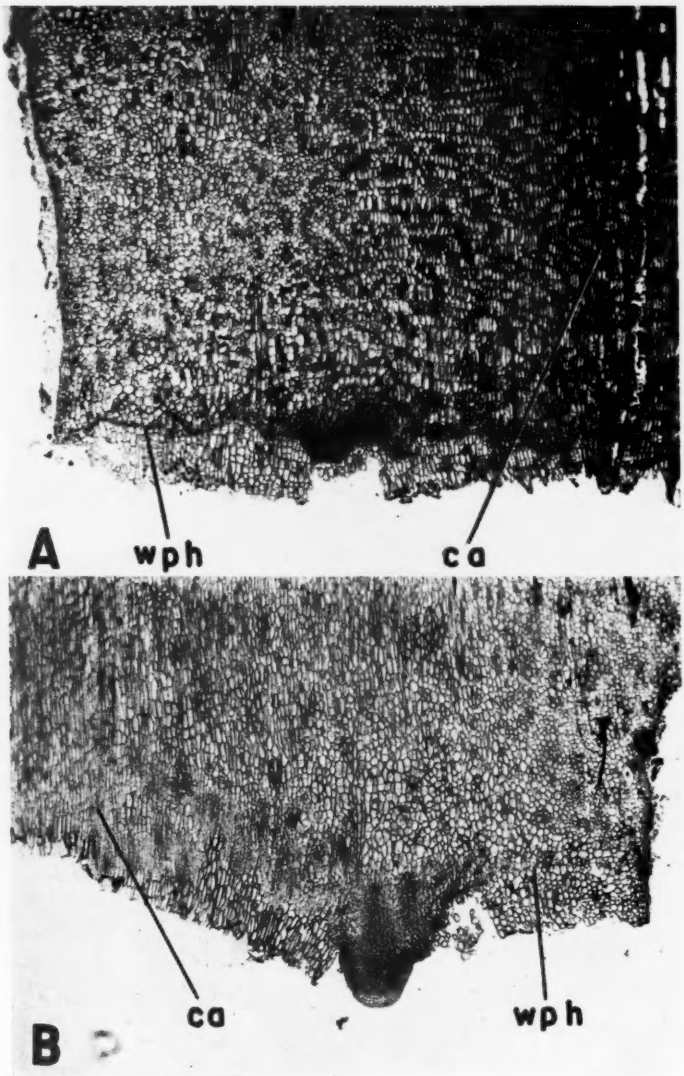

FIG. 11.—Sections through lower cut surface of treated segments. *A:* 10 days after treatment. Secondary phloem parenchyma deeper in from cut surface becoming involved in formation of root primordia. *B:* 13 days after treatment. A root is becoming organized and some cells between developing root and cambium are differentiating into vascular elements. *ca*, cambium; *wph*, wound phellogen.

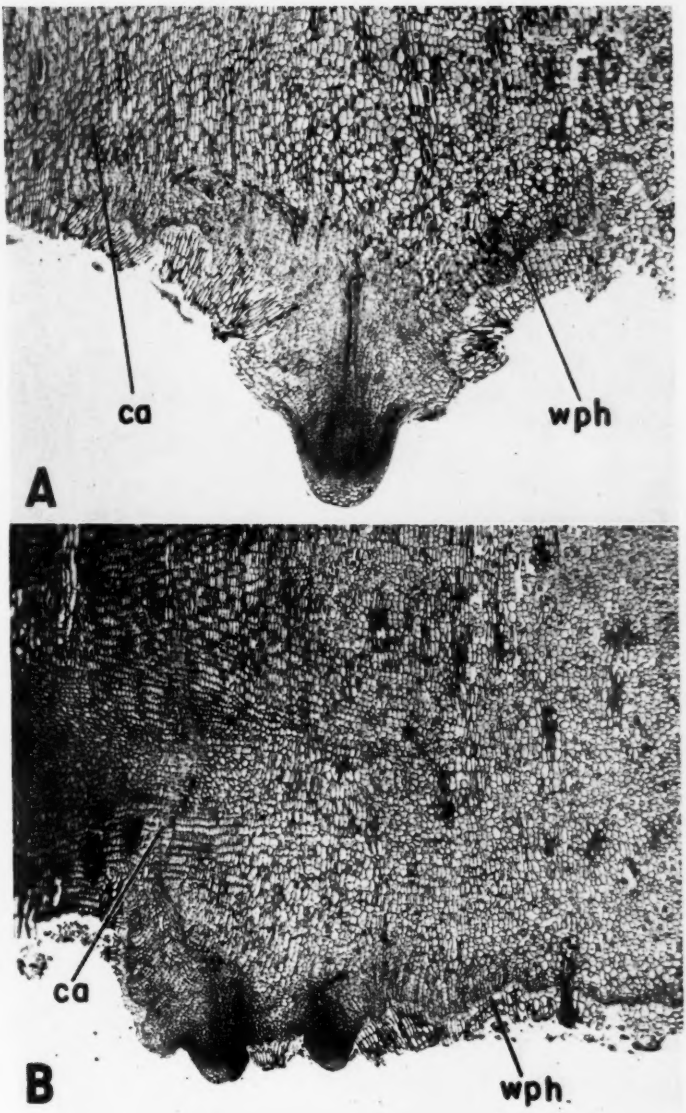

FIG. 12.—Sections through lower cut surface of segment 14 days after treatment. Cells lying between developing roots and cambium differentiate into vascular elements and establish vascular connections. *ca*, cambium; *wph*, wound phellogen.

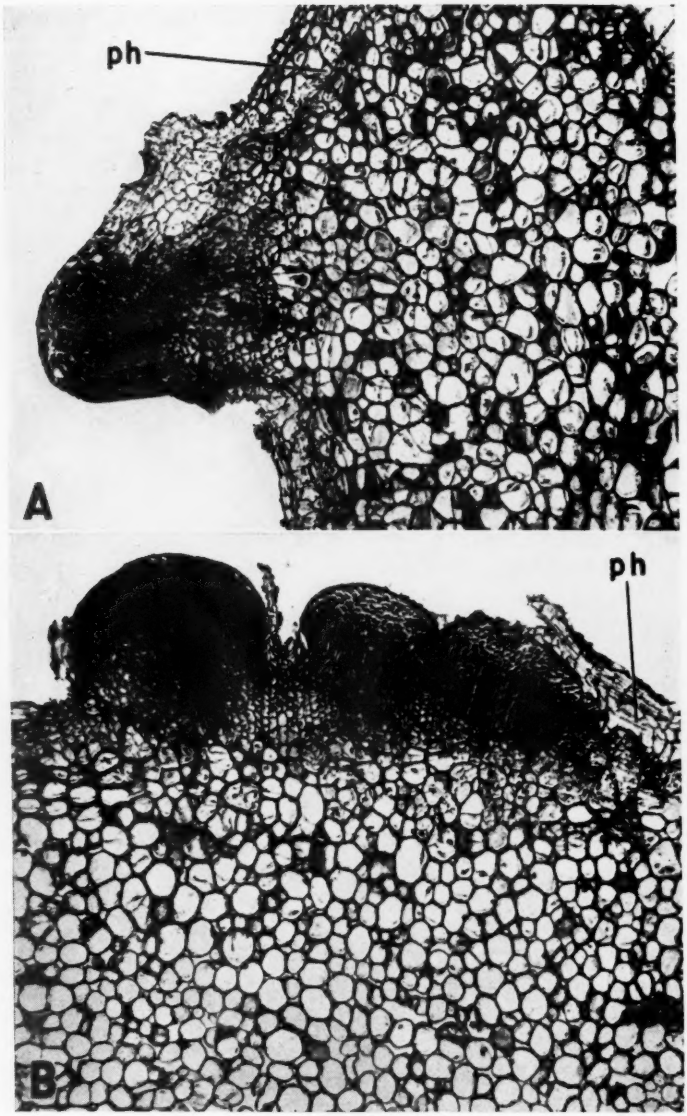

FIG. 13.—Longitudinal sections through outer portions of segment 14 days after peripheral treatment. Derivatives of phellogen, pericycle, and secondary phloem parenchyma form root primordia that rupture the periderm. Outer cells of developing roots dying; little cellular differentiation at base of root primordia (*cf.* figs. 11, 12). *ph*, phellogen.

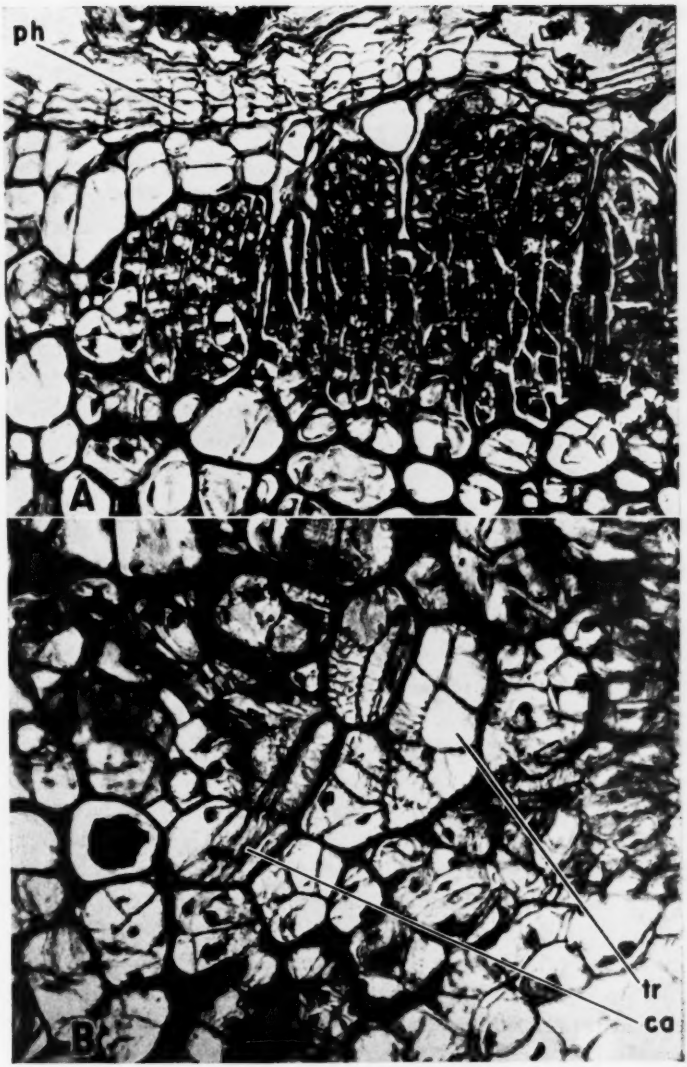

FIG. 14.—Longitudinal sections through outer portions of segments 14 days after lateral treatment. *A:* intense activity of derivatives of thick walled parenchymatous cells within parent cell walls. *B:* differentiation of derivatives of thick walled parenchymatous cells at base of developing root into tracheids and cambial elements. Four tracheids within parent cell wall. *ph*, phellogen; *tr*, tracheid; *ca*, cambial cell.

parenchymatous cells just inside the periderm are usually thick walled. Storage cells containing myrosin are found in both the phloem and xylem regions.

Lateral roots are derived from the pericycle in the very young root before much cambial activity has occurred (figs. 2, 3). The endodermis is not involved in lateral root formation, but is crushed very early by the developing root. After a phellogen has appeared in the outer layers of the pericycle, additional lateral roots develop only in association with a root trace.

DEVELOPMENT OF ROOTS AND BUDS FROM LATERAL ROOT TRACES.—In the control segments, the origin of roots and buds is always in close association with a lateral root trace. The first discernible activity is seen in the cells closely associated with the phellogen and the cambium of the lateral root trace. The phellogen and its immature derivatives are involved as well as the derivatives of the cambium (fig. 6*A*). Soon the xylem and phloem parenchyma of the lateral root trace and associated parenchymatous cells of the main root begin active division and a mass of meristematic tissue is formed which, as a result of its growth and enlargement, ruptures the periderm (fig. 6*B*). If the lateral root trace is situated at the morphological lower end of the segment, organization into a root proceeds with typical histogen formation. This organization is usually complete before the periderm is ruptured. Differentiation of cells in the developing root proceeds back to the lateral root trace, so that the phloem and xylem of the new root and those of the lateral root trace are connected. On the other hand, if the lateral root trace is situated at the morphological upper end of the segment, a bud may be organized instead of a root. The first distinguishable region of the bud is a well defined dermatogen, which is followed by the formation of leaf primordia through differential activity of cells in the meristem (fig. 6*C*). Differentiation of vascular elements takes place between the developing bud and the root trace (fig. 6*D*). At the base of a bud, a region in close association with the epidermis of the bud and the phellogen of the segment often becomes active. This activity later involves cortical parenchyma at the base of the bud and a root primordium is organized. Differentiation of vascular elements in the cells between the root primordium and the bud takes place, thus establishing vascular connections between them.

Organization of both roots and buds usually begins about 5 or 6 days after the segment is treated with pure lanolin. Some meristematic areas appear sooner than others, however, and various stages of development may be found around any single root trace. Certain areas may be more or less meristematic in any intact fleshy root; this activity is dependent upon the past environmental conditions of the root.

When 2 per cent naphthylacetic acid in lanolin is applied to the upper cut surface of a segment, only roots are organized in the lateral root trace areas. With indoleacetic acid a few buds may be formed, but not nearly so many as in the controls. The same regions seem to be involved in bud and root formation, the only difference being in the subsequent organization of the mass of meristematic tissue (fig. 7). Organization of roots takes place about 5 or 6 days after the segment is treated, the time relations thus being about the same as in the control segments.

DEVELOPMENT OF CUT SURFACES.—During the first 2 days the cut surfaces of the control segments exhibit no apparent changes except for the deposition of material on the cell walls along the surface. Cell divisions first appear in the young secondary phloem, five or six cell layers in from the lower cut surface. Within 4 days a well defined wound phellogen has formed across the entire lower cut surface. In addition there is some callus formation, owing mainly to cell elongation, although some cell divisions may occur. Callus production ceases on about the eighth day. The phellogen becomes comparatively inactive about this time also, but the cells of the callus remain alive for some time after the death of cells beneath them (fig. 9*A*). After about 10 days the wound phellogen is complete across the upper part of the segment (fig. 8*A*). Necrotic areas are formed at both cut surfaces, extending deeply into the xylem and phloem, and these increase in extent until the wound periderm is formed. Since the formation of a phellogen near the upper cut surface does not take place so rapidly as it does near the lower cut surface, the necrotic areas are much more extensive near the upper than near the lower surface.

If the segment includes a lateral root trace near the upper surface, meristems may develop in the region where the cambium of the root trace comes in close proximity to the wound phellogen. These

meristems develop only in those regions where the root trace extends through the cambial region and the phloem. None has been observed in association with the portion of the trace which extends through the xylem. If the lateral root trace occurs near the lower cut surface, the meristems will give rise to roots, while if the trace occurs near the upper cut surface, the meristems may differentiate as buds.

After the application of 2 per cent naphthylacetic acid, the cells near the upper cut surface follow the same general pattern of response as was found in the control segments, except that the death of cells takes place much more slowly (fig. 8*B*). The treatment, however, induces a much greater response in the cells near the lower surface. The wound phellogen, instead of becoming comparatively inactive after 8 days, proceeds to divide very actively, and adjacent cells also become active. Meristems, that later give rise to roots, originate from certain of these active regions. During the first 8 days the activity of the phellogen is confined chiefly to the phloem region (fig. 9*B*). By the tenth day, however, areas of wound phellogen formation may be found in the xylem. This is in contrast to the control, in which the phellogen extended across the surface at the end of 8 days. The xylem activity varies considerably. In some segments a well developed phellogen may develop across the entire xylem area, while in others it may be localized.

Those roots which develop from meristematic areas not associated with a lateral root trace are derived from phloem parenchyma, xylem parenchyma, and cambial regions. Even myrosin storage cells may be involved (fig. 10*B*). Roots arise most frequently in the phloem and cambial regions. Root organization from these meristems is first observed about 12 days after treatment. After the development of histogens in the root, differentiation of vascular elements takes place between the root and the main cambial region (figs. 11, 12). Differentiation of scattered phloem and xylem elements and scattered secondary cambiums may take place in active regions not in association with a developing root (fig. 10*A*). The roots from the xylem parenchyma usually originate near a strand of tertiary phloem.

Treatment of the cut surfaces also induced the differentiation

of more vessels, sieve tubes, and companion cells from the derivatives of the main cambium, with consequent reduction in the relative number of new phloem and xylem parenchyma cells.

In both control and treated segments, a vessel that abuts the cut surface may be crossed by the wound phellogen. The dividing cells on either side of the vessel force their way into its lumen through pits. There they divide and enlarge until the phellogen is complete across the vessel. Divisions may proceed until the lumen is partially filled with these cells and has the appearance of a vessel with tyloses.

RESPONSE TO LATERAL TREATMENT.—The first noticeable activity as a result of lateral application of indoleacetic or naphthylacetic acids is in the phellogen and the adjacent five or six parenchymatous layers. Later these inner cells become more active, and they may either form localized phellogens, resulting in the death of cells exterior to them, or continue to divide without forming a phellogen (fig. 14*A*). In the latter case organization of the cells into roots may occur (fig. 13). Organization may take place in the outermost parenchymatous layers, or it may take place ten or fifteen cell layers in from the phellogen, and orientation of the new root axis may be in any direction. These roots do not develop further but soon die. A few tracheids and cambial elements may be differentiated at the base of the partially developed roots.

Discussion

The horseradish root has frequently been used for studying regenerative phenomena in plants. Years ago RECHINGER (**21**) described the gross development of roots and buds from the regions of the root traces. He found that transverse sections of the root only 1.5 mm. thick were capable of producing buds. Later KUPFER (**14**) repeated this work and in some instances claimed to have obtained buds from the cut surfaces. It seems likely that these buds developed from root traces near the cut surface, for PRIESTLEY and SWINGLE (**20**) could not confirm KUPFER'S work, nor could the present writer. If segments of the horseradish root are cut near a lateral root trace, buds will develop in association with the root trace near the upper surface and break through, thus giving the appearance that they actually arise from the surface.

BEALS (2) did a limited amount of histological work with the horseradish root and stated that buds developed from cambial cells. This was not found to be strictly the case in the material presented here, although it is true that cambial derivatives may be involved in root and bud production. The development of roots from the cut surfaces is similar to that described by PRIESTLEY and SWINGLE (20) for untreated segments of sea kale root.

It appears that the application of either indoleacetic or naphthylacetic acid, in the relatively high concentrations used here, inhibits the organization and development of buds but stimulates the organization and development of roots. Similar results have recently been reported for sea kale roots (23). This is in agreement with most recorded work (4, 25). GREENLEAF (8), however, working with *Nicotiana*, found stimulation of shoot production from the cut surfaces of decapitated stems when they were treated with 1 per cent indoleacetic acid in lanolin. He found it necessary to remove all lateral buds in order to obtain adventitious buds from the callus. GOLDBERG'S (7) studies show that the application of indoleacetic acid to the stems of decapitated cabbage seedlings stimulates the production of roots and buds from the callus. She did not remove the lateral buds. Decapitated plants, when left untreated, produced comparatively few buds from the callus. BEAL (1) reports the development of buds in the leaf axis of *Lilium harrisii* when the stem is decapitated and the cut surface is treated with 3 per cent indoleacetic acid in lanolin. Untreated plants do not produce axillary buds. In this case there is no callus formed in the region of bud development. With the same treatment two other species of lily formed roots but not buds. LINK and EGGERS (16) found that the application of relatively high concentrations of indoleacetic acid to the decapitated hypocotyls of flax plants inhibited the formation of hypocotyledonary buds. They further report (17) that in certain low concentrations there is a stimulation of bud development. The interpretation of these varying results remains for the future.

When growth substances were applied to either the morphological upper or lower surfaces of the horseradish root segments, roots developed from the lateral surfaces and from cells adjacent to the lower cut surface, but never from the upper cut surface. Yet

when the growth substances were applied peripherally, roots developed from the outer parenchymatous cells in the region of application near the upper surface. Other workers have shown that when the stems of various plants are treated with relatively high concentrations of auxin-like compounds, numerous adventitious roots are produced near the treated regions (**1, 3, 6, 7, 9, 10, 11, 13, 15, 22**). When the whole fleshy axis of the horseradish plant was cut longitudinally and the cut surfaces treated, the development of roots from the lateral root traces was confined to two areas: one composed of the stem proper and the root-stem junction, and the other at the extreme lower end of the root. In the cases where the whole fleshy axis was cut transversely into 4 cm. segments, the extreme lower end responded the same as did the majority of the root segments, while the segment that contained the stem region was by far the most active in both root and shoot production. Although it is possible that the activity in the upper part of the fleshy structure was related mainly to the much greater number of traces coming to the surface there, many other factors undoubtedly also played a role.

The response of the root was much more intense when the growth substances were applied to the upper cut surfaces of the segments than when they were applied to the lower cut surfaces. This would indicate that the substances travel downward through the segment much more readily than they do upward. These observations are in accord with the work of previous investigators (**4, 25**). If this is true, the comparatively small amount of meristematic activity in the xylem of the lower portions of the segments might be explained on the basis of the accumulation of the growth substances (and any other compounds through which they might act) in the phloem and cambial regions, thus making the xylem comparatively low in these substances. This is further substantiated by the fact that cellular activity was most intense in the regions of the xylem where tertiary strands of phloem were developed.

Either the main phellogen of the root or a wound phellogen was involved in all cases of root and shoot production in the mature root. Formative activity was usually greatest in the region of the cambium of the lateral root trace. Meristems, resulting in the production of roots from phloem and xylem parenchyma, were formed only after

wound phellogens had been produced. Derivatives of these phellogens made up the outer portions of the developing roots. In the control segments, roots and buds were formed readily from the regions where the lateral root traces approached a phellogen, but without root traces there was no formation of buds and only an occasional root from the cut surfaces.

Naphthylacetic acid was much more effective than indoleacetic acid in producing response in the horseradish. Its superiority may be due to its greater stability or to its greater mobility (or both) within the plant. Peripheral application of naphthylacetic acid is more effective than application at the lower cut surface. Indoleacetic acid, when applied to the bottom of the segment, is more effective than when applied peripherally. Distribution of these compounds from a lateral application would necessitate their movement through the phellem to the phellogen, and then either down the phellogen or outer parenchymatous cells, or through a great mass of phloem parenchyma cells to the sieve tubes. Apparently naphthylacetic acid is more mobile through these tissues than is indoleacetic acid.

When the segments were washed with water for 24 hours before being treated, no callus was formed. This would tend to indicate either the loss of some building materials such as amino acids and sugars, a more specific substance like tyrosine (**18**), or even more intricate substances that control the formation of callus similar to (or identical with) the "wound hormones" reported by several workers (**5, 24**). These results suggest that the application of indoleacetic and naphthylacetic acids did not cause callus formation directly, but merely affected the ditribution of some controlling substances. The greatly increased susceptibility of the washed segments to invasion by micro-organisms may indicate that some substance or substances which give some degree of immunity were leached out. There remains, of course, the possibility that the segments were more susceptible to invasion during the 24 hours they were in the water.

In both the control and treated segments, cellular differentiation into vascular elements (in the region of lateral root traces) took place only after roots and buds were well formed. Then vascular connec-

tions between the developing root or shoot and the lateral root trace were differentiated. When roots developed from the lower cut surface of treated segments, differentiation proceeded between the developing root and the cambium. However, certain active areas not involved in root production differentiated small scattered strands of phloem and xylem elements and secondary cambiums. Peripheral application of the growth substances produced meristematic areas so far away from the cambium that cellular differentiation into vascular elements occurred in localized areas at the base of a developing root, but further centripetal differentiation did not occur. These roots soon perished, probably from lack of vascular connections.

Summary

1. In the young horseradish root, lateral roots develop from the pericycle or its derivatives, resulting in crushing of the endodermis.

2. In the mature root, or transverse segments of it, buds originate in the region where the cambium of the upper root traces comes in contact with the phellogen. Roots originate from lower root traces in a similar manner. Derivatives of the cambium, phellogen, pericyclic parenchyma, phloem parenchyma, and xylem parenchyma may all be involved in the development of buds and roots. Occasionally a root may be formed from the lower cut surface without a root trace being involved.

3. Application of relatively high concentrations of indoleacetic and naphthylacetic acids inhibits shoot production and stimulates root production. Under the influence of these compounds, roots are organized in the regions where buds would otherwise have organized. In addition, the morphologically lower cut surfaces of the treated segments produce numerous roots from derivatives of the wound phellogen, phloem parenchyma, xylem parenchyma, and cambium. There is no root organization from the upper cut surface unless a lateral root trace is involved.

4. Peripheral application of indoleacetic or naphthylacetic acid induces activity in the outer parenchymatous cells of the root, which culminates in the formation of new roots that soon perish.

5. Placing the cut segments in running water for 24 hours before

treatment inhibits the production of callus and results in an increased invasion by micro-organisms.

6. Various extracts of the horseradish plants were ineffective in the stimulation of the production of buds.

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CYTOLOGICAL STUDIES IN RELATION TO THE CLASSIFICATION OF THE GENUS CALOCHORTUS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 498

J. M. BEAL

(WITH FORTY FIGURES)

Introduction

The species of *Calochortus* are common and widespread liliaceous plants of the Pacific Coast. They range from Canada to Guatemala and eastward to Nebraska and the Dakotas. Within these limits no considerable region is without one or more species. The genus is found naturally only in western North America, with a greater number of species present in California than in any other similar area.

Not only is the range of the genus extensive but that of several of the species is great, and the conditions of soil and climate under which they grow show great variation. Under such diverse environmental conditions it is perhaps natural to expect considerable variation in many of the species, and this has been repeatedly observed by those who have collected the plants in their natural habitats. Variation has been equally apparent under garden conditions. According to PURDY (private correspondence with the writer), hybridization appears to be of infrequent occurrence. In general, the various species produce seeds in abundance. The genus has proved to be a difficult one for systematists, and there is still question concerning the determination and taxonomic arrangement of many of the species.

The present investigation shows that the number and morphology of the chromosomes are characteristics which aid in determining the natural relationships among the species. No attempt will be made to review all the taxonomic literature pertaining to the genus, but

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three treatments which have some bearing upon the cytological findings will be briefly mentioned.

In 1879, WATSON (7) divided the genus into three sections, Eucalochortus, Mariposa, and Cyclobothra, placing eleven species under the first section, fourteen under the second, and six under the third. The third section comprises the Mexican species, while the first and second include those found north of Mexico. One species, *C. catalinae*, was unassigned because the flowers were unknown to him.

The second is that of PURDY (6), published in 1901. Only species occurring north of Mexico are considered in his monograph. These are divided into the Eucalochortus and Mariposa sections, essentially as was done by WATSON. PURDY includes sixteen species under the first section and twenty-four under the second, making a total of forty species as compared with WATSON'S twenty-five. The group arrangement under the sections differs somewhat in the two treatments, but their criteria for sectional distinction are essentially the same.

The third treatise is that of ABRAMS (1), published in 1923. He makes no formal separation of the genus into sections. The key to the species is arranged in two main divisions: capsule winged on the angles and capsule not winged. All the species examined by the writer which possess 20 (or 40) somatic chromosomes fall into the first category, while those which show some other number fall into the second. The capsule may perhaps furnish the most significant morphological structures in the genus.

Although Index Kewensis lists seventy-nine species for the genus, it is generally agreed among those American taxonomists who have worked most carefully with the group that there are approximately fifty valid species.

NEWTON (5) has reported the number and discussed briefly the chromosome morphology in the following ten species:

EUCALOCHORTUS	2n	n
C. albus Dougl.	20	10
C. amabilis Purdy	20	10
C. benthami Baker	20	10
C. maweanus Leicht	20	

MARIPOSA		
C. plummerae Greene	18	
C. clavatus S. Wats.	16	
C. catalinae S. Wats.	14	7
C. luteus Douglas	14	
C. venustus var. *eldorado* Purdy	14	7
C. vesta Purdy	28	14

All these species except *C. benthami* have been examined by me and the numbers reported by NEWTON confirmed. All the available material of *C. luteus* has shown a somatic number of 20 or 21 chromosomes, indicating that it was triploid, especially as two varieties of the species, *C. luteus* var. *citrinus* and *C. luteus* var. *oculatus*, both possess a diploid number of 14 chromosomes. NEWTON observed the striking similarity of size and form variations among the chromosomes of the species assigned to certain groups, as well as the occurrence of certain forms in all the species. This condition has been observed during the present study.

Material and methods

Although the material has been obtained from several sources, bulbs of most of the species and varieties investigated were secured from MR. CARL PURDY, Ukiah, California, whose determinations in the main have been accepted by the writer. *C. gunnisoni* and *C. nuttallii* were secured from MR. CLAUDE A. BARR, Smithwick, South Dakota. MR. MARION OWNBEY, Missouri Botanical Garden, kindly sent identified material of *C. luteus*, *C. elegans*, and *C. selwayensis*, and MISS VADA H. ALLEN, Department of Botany, University of Idaho, supplied bulbs of *C. elegans*. *C. aureus* was also obtained from MR. RALPH O. BAIRD, Gallup, New Mexico. To all these collaborators the writer expresses his gratitude.

The bulbs were potted during the autumn in a sandy loam soil and placed in a coldframe until freezing temperatures occurred, when they were placed in one of the cooler rooms of the greenhouses. As roots began to emerge their tips were cut off, and in most instances, were fixed in both LaCour's 2 BE and Navashin's solutions. On the whole, LaCour's fixative gave the better results, and most of the figures are from slides fixed in this solution. After fixation, the ma-

terial was run up through the usual schedule and imbedded in paraffin. Transverse sections were cut at 20–25 μ in thickness and stained according to the gentian violet-iodine method.

A number of the species produced flower buds from which anthers were taken for the study of meiosis. To determine the stage in meiosis, one anther from each bud examined was crushed in aceto-carmine. When appropriate stages were found, the remaining anthers were immediately smeared on a clean slide, fixed for two hours in Navashin's solution, and stained with the gentian violet-iodine stain. For comparison with the somatic chromosomes at comparable stages, only figures of the first meiotic metaphases during microsporogenesis are shown.

All drawings were made at table level with the aid of a camera lucida, using a 15× compensating ocular and a 90× apochromatic objective, n.a. 1.40, with a yellow-green filter. The magnification is approximately 2450.

Observations and taxonomic arrangement

The arrangement of the sections and subsections presented in the following classification is that supplied by MR. MARION OWNBEY, who is engaged in a monographic study of the genus at the Missouri Botanical Garden. The chromosome numbers of all the species and varieties here included were sent to him during the autumn of 1938. For some of these species he had determined the numbers, and in addition had made counts on some not listed in this paper. In all cases our determinations have been in agreement and the classification herewith presented appears to represent a natural arrangement based upon both cytological and morphological relationships. It is a pleasure to acknowledge my indebtedness to MR. OWNBEY for his kindness in furnishing the classification and permitting it to be used here before he publishes it in its final and more complete form.

SECTION I. EUCALOCHORTUS

Under section I are included all species and varieties which possess 10 as the basic number of chromosomes and which have capsules winged on the angles. This necessitates the transfer to section I of those species which PURDY (6) places under section II as the group

Oregon Mariposas. Four of the five species listed by PURDY for the group possess 10 as the basic number of chromosomes. A fifth species, *C. lyallii*, mentioned by PURDY as a synonym for *C. elegans* var. *nanus* (which it is not), has a like number. These species also have ovate, three-winged capsules, and therefore are placed under subsection I D, Nitidi, forming a natural and coherent group on both cytological and morphological grounds.

Subsection I A. Eleganti

C. tolmiei Hooker & Arnott.—According to OWNBEY, *C. tolmiei* should include *C. maweanus* Leicht. and its varieties. Three varieties of *C. maweanus* were secured from PURDY, var. *major* Purdy, var. *purpurascens* Purdy ined., and var. *roseus* Purdy. All possess the same number of chromosomes, and their karyotypes are so similar as to be indistinguishable. For this reason only one of them, var. *major*, is illustrated, and this has a diploid number of 20 chromosomes (fig. 1) with 10 pairs at metaphase I of meiosis (fig. 1*a*).

C. elegans Pursh.—Diploid, 20 chromosomes (fig. 2). Karyotype similar to preceding.

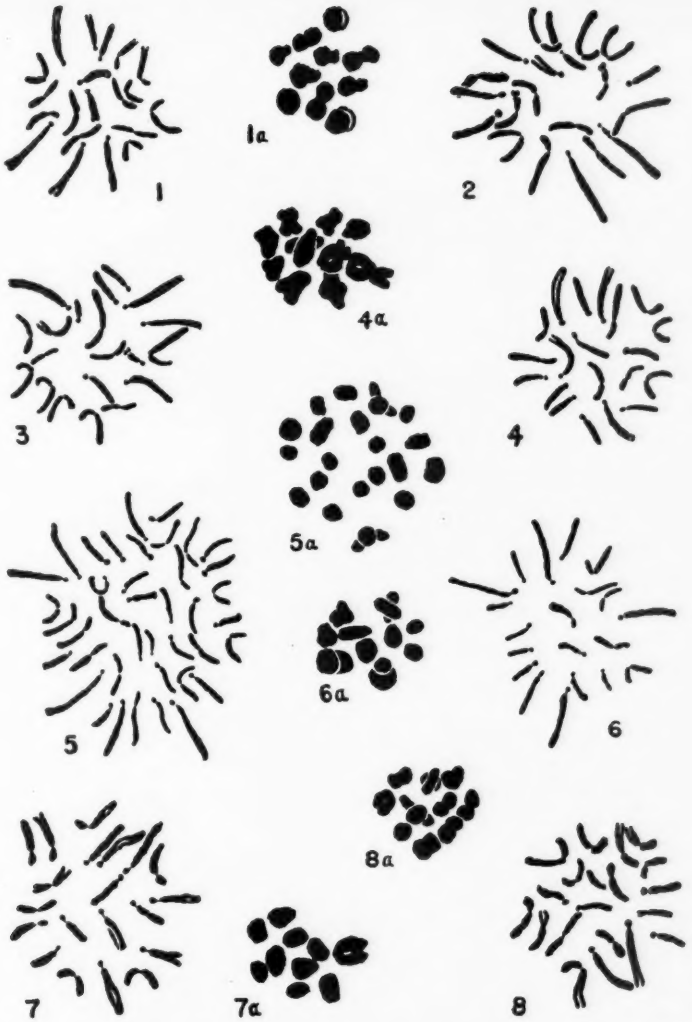
C. selwayensis St. John.—Diploid, 20 chromosomes (fig. 3). Karyotype scarcely distinguishable from that of *C. elegans*, of which it may be merely a variety.

C. apiculatus Baker.—Diploid, 20 chromosomes (fig. 4); 10 pairs at metaphase I of meiosis (fig. 4*a*). The karyotype of this species differs slightly from those of the preceding three. The differences may perhaps result from a certain amount of translocation or inversion, or a combination of both processes.

Subsection I B. Nudi

C. uniflorus Hooker & Arnott (*C. lilacinus* Kellogg).—Diploid, 40 chromosomes (fig. 5); 20 pairs at metaphase I of meiosis (fig. 5*a*). The various forms of chromosomes occur in quadruplicate and hence this species is a tetraploid. Pairing appears to be regular in meiosis with no evidences of multivalent association. There is some indication of secondary association in figure 5*a* however.

C. nudus S. Wats.—Diploid, 20 chromosomes (fig. 6). Karyotype strikingly similar to that of the preceding species, with the various forms of chromosomes present in both.



FIGS. 1–8.—Fig. 1, *C. tolmiei*, root tip metaphase. Fig. 1*a*, same, metaphase I. Fig. 2, *C. elegans*, root tip metaphase. Fig. 3, *C. selwayensis*, root tip metaphase. Fig. 4, *C. apiculatus*, root tip metaphase. Fig. 4*a*, same, metaphase I. Fig. 5, *C. uniflorus*, root tip metaphase. Fig. 5*a*, same, metaphase I. Fig. 6, *C. nudus*, root tip metaphase. Fig. 6*a*, same, metaphase I. Fig. 7, *C. amabilis*, root tip metaphase. Fig. 7*a*, same, metaphase I. Fig. 8, *C. amoenus*, root tip metaphase. Fig. 8*a*, same, metaphase I.

Subsection I C. Pulchelli

C. amabilis Purdy.—Diploid, 20 chromosomes (fig. 7); 10 pairs at metaphase I of meiosis (fig. 7*a*).

C. amoenus Greene.—Diploid, 20 chromosomes (fig. 8); 10 pairs at metaphase I of meiosis (fig. 8*a*).

C. albus Douglas.—Diploid, 20 chromosomes (fig. 9); 10 pairs at metaphase I of meiosis (fig. 9*a*).

The karyotypes of the three species embraced under this subsection are so similar that it would be practically impossible to distinguish them from one another, except for the fact that *C. albus* has one pair of chromosomes with attachment constrictions so elongated as to similate, if not actually to constitute, a satellited condition.

Subsection I D. Nitidi

C. lyallii Baker.—Diploid, 20 chromosomes (fig. 10); 10 pairs at metaphase I of meiosis (fig. 10*a*).

C. howellii S. Wats.—Diploid, 20 chromosomes (fig. 11); 10 pairs at metaphase I of meiosis (fig. 11*a*).

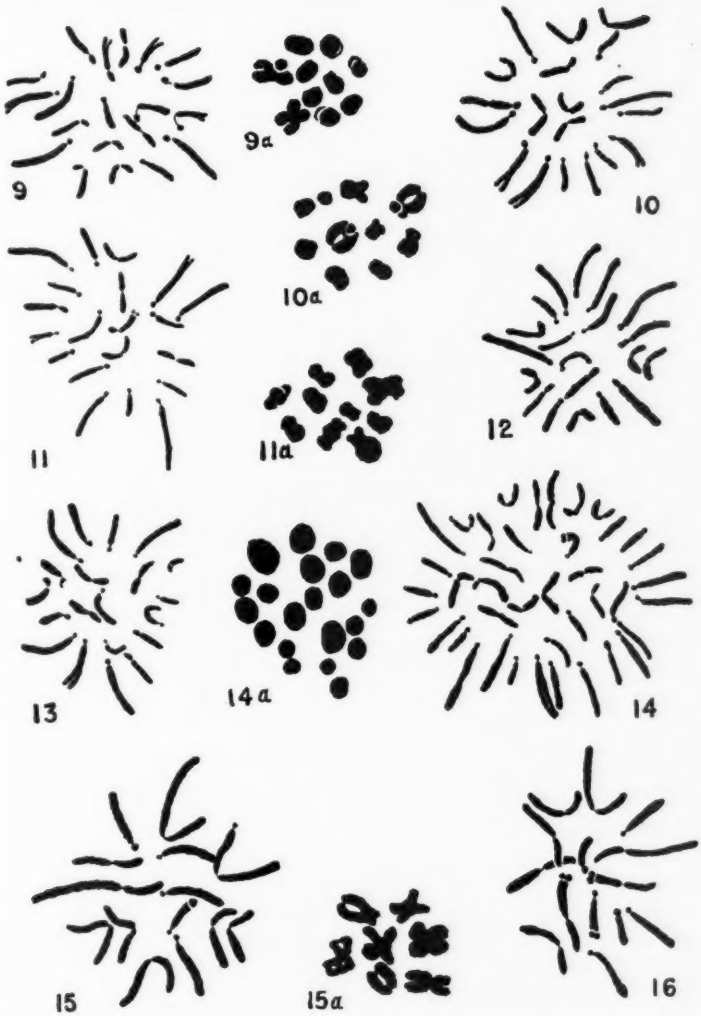
C. nitidus Douglas (*C. eurycarpus* S. Wats.).—Diploid, 20 chromosomes (fig. 12).

C. greenii S. Wats.—Diploid, 20 chromosomes (fig. 13).

C. pavonaceus Fernald (*C. nitidus* of several authors, not of Douglas).—Diploid, 40 chromosomes (fig. 14); 20 pairs at metaphase I of meiosis (fig. 14*a*). As in *C. uniflorus*, the various types of chromosomes occur in quadruplicate and this is also a tetraploid. PURDY placed this species, together with *C. nitidus* (*C. eurycarpus*), *C. greenii*, and *C. howellii*, under the section Mariposa and the group Oregon Mariposas, and stated it was "the largest flowered of the group." Because of the chromosome numbers and the winged capsules it is evident that all these species belong under the section Eucalochortus.

SECTION II. MARIPOSA

This section comprises those species with a basic number of chromosomes other than 10, and which have capsules not winged. The section is decidedly more diverse in the number of chromosomes and karyotypes than is section I. Somatic numbers of 12, 14, 16,



FIGS. 9–16.—Fig. 9, *C. albus*, root tip metaphase. Fig. 9*a*, same, metaphase I. Fig. 10, *C. lyallii*, root tip metaphase. Fig. 10*a*, same, metaphase I. Fig. 11, *C. howellii*, root tip metaphase. Fig. 11*a*, same, metaphase I. Fig. 12, *C. nitidus*, root tip metaphase. Fig. 13, *C. greenii*, root tip metaphase. Fig. 14, *C. pavonaceus*, root tip metaphase. Fig. 14*a*, same, metaphase I. Fig. 15, *C. catalinae*, root tip metaphase. Fig. 15*a*, same, metaphase I. Fig. 16, *C. splendens*, root tip metaphase.

18, 20, 21, 28, and 32 chromosomes have been observed, the numbers 28 and 32 being from tetraploids and 20 and 21 from triploids.

Subsection II A. Venusti

C. catalinae S. Wats.—Diploid, 14 chromosomes (fig. 15); 7 pairs at metaphase I of meiosis (fig. 15*a*). One or both of the long medianly constricted chromosomes may possess satellites.

C. splendens Douglas.—Diploid, 14 chromosomes (fig. 16).

C. splendens var. *rubra* Purdy.—Diploid, 14 chromosomes (fig. 17); 7 pairs at metaphase I of meiosis (fig. 17*a*). Karyotype scarcely distinguishable from the species.

C. leichtlinii Hooker.—Diploid, 14 chromosomes (fig. 18). Chromosomes somewhat shorter than in preceding species but otherwise similar.

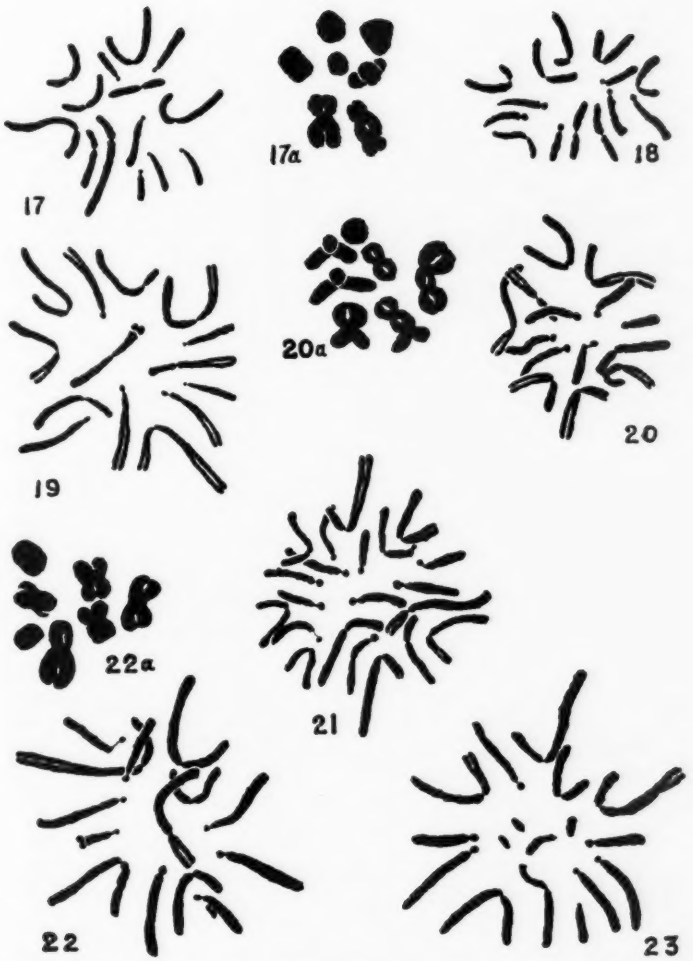
C. venustus Douglas.—Diploid, 14 chromosomes (fig. 19). This and the succeeding species of the subsection have somewhat longer and larger chromosomes than any of the other species examined.

C. venustus var. *caroli* Cockerell.—Diploid, 14 chromosomes (fig. 20); 7 pairs at metaphase I of meiosis (fig. 20*a*). One bulb was triploid, showing 21 chromosomes in its root tip cells (fig. 21). Karyotypes similar to that of the species.

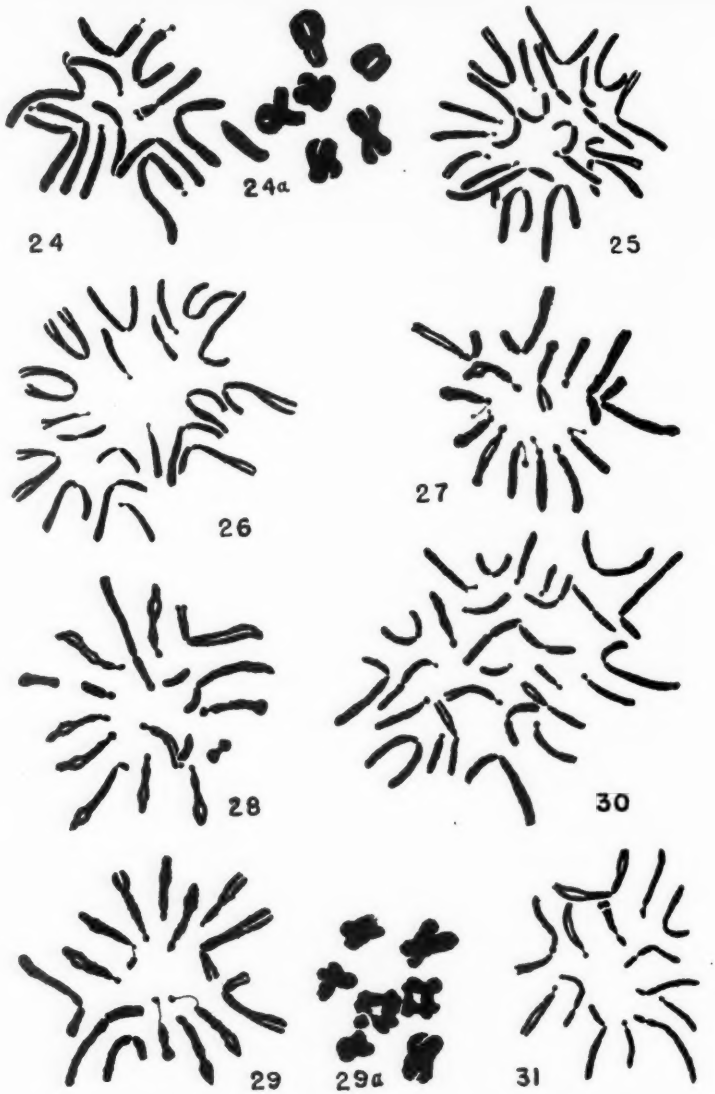
C. venustus var. *purpurascens* S. Wats.—Diploid, 14 chromosomes (fig. 22); 7 pairs at metaphase I of meiosis (fig. 22*a*). Fragmentation appears to be of frequent occurrence, as a number of the preparations showed a condition similar to that of figure 23. But neither the bulbs nor the plants produced from them showed external evidences of abnormalities.

C. superbus Purdy.—Diploid, 12 chromosomes (fig. 24); 6 pairs at metaphase I of meiosis (fig. 24*a*). HOWELL (2) states that "*C. superbus* seems most closely related to *C. luteus*." It seems probable that *C. superbus* may have been derived from *C. luteus* through fusion and translocation between two of its chromosomes, designated as chromosomes 2 and 6 (figs. 39, 40), with elimination of the attachment constriction of chromosome 2. This possibility is discussed in greater detail in a later section.

C. luteus Douglas.—NEWTON reported the diploid number for this species as 14, but all material available to me from both PURDY and



FIGS. 17–23.—Fig. 17, *C. splendens* var. *rubra*, root tip metaphase. Fig. 17*a*, same, metaphase I. Fig. 18, *C. leichtlinii*, root tip metaphase. Fig. 19, *C. venustus*, root tip metaphase. Fig. 20, *C. venustus* var. *caroli*, root tip metaphase. Fig. 20*a*, same, metaphase I. Fig. 21, same, root tip metaphase from triploid bulb. Fig. 22, *C. venustus* var. *purpurascens*, root tip metaphase, one fragment present. Fig. 23, same, root tip metaphase, several fragments present.



FIGS. 24–31.—Fig. 24, *C. superbus*, root tip metaphase. Fig. 24*a*, same, metaphase I. Fig. 25, *C. luteus*, root tip metaphase, 21 chromosomes. Fig. 26, same, root tip metaphase, 20 chromosomes. Fig. 27, *C. luteus* var. *citrinus*, root tip metaphase. Fig. 28, same, root tip metaphase showing fragments. Fig. 29, *C. luteus* var. *oculatus*, root tip metaphase. Fig. 29*a*, same, metaphase I. Fig. 30, *C. vesta*, root tip metaphase. Fig. 31, *C. macrocarpus*, root tip metaphase.

OWNBEY has shown either 20 (fig. 25) or 21 (fig. 26) chromosomes in root tip cells, with the latter number occurring far more commonly. The various chromosome forms occur in triplicate, and there is therefore every reason to consider this material to be triploid. No meiotic material has been available and it has not been possible to observe the behavior of the chromosomes during the meiotic prophases. OWNBEY collected apparently normal seeds produced from some of the bulbs examined, and apparently seed production is common in this triploid form.

C. luteus var. *citrinus* (Baker) S. Wats.—Diploid, 14 chromosomes (fig. 27). Fragmentation appears to be common in this variety also (fig. 28). Five chromosomes in figure 27 show satellites, possibly as the result of translocated fragments.

C. luteus var. *oculatus* S. Wats.—Diploid, 14 chromosomes (fig. 29); 7 pairs at metaphase I of meiosis (fig. 29*a*). Two chromosomes in figure 29 show satellites and a third an attached fragment.

C. vesta Purdy.—Diploid, 28 chromosomes (fig. 30). This tetraploid form is placed as a variation or color form of *C. venustus* by some taxonomists, but it appears to merit specific rank on both cytological and morphological grounds. Its chromosomes resemble those of *C. luteus* more closely than those of *C. venustus*, and apparently it is more closely related to the former than to the latter.

Subsection II B. Macrocarpi

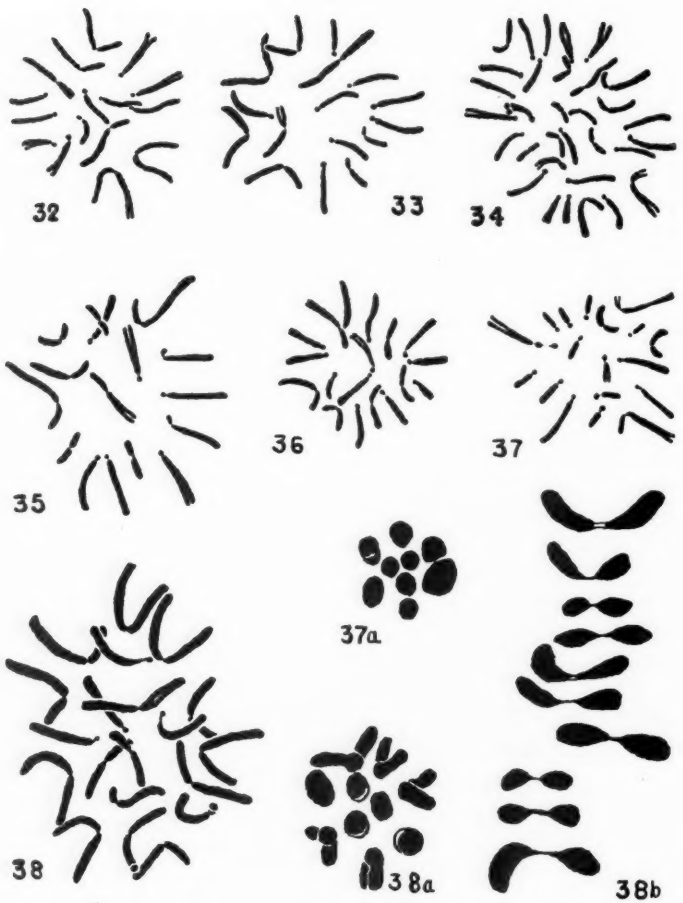
C. macrocarpus Douglas.—Diploid, 14 chromosomes (fig. 31). This species appears to combine some characters of the preceding subsection with some of the following subsection, but differs from either in certain other respects. And while it is probably more closely related to subsection II C on the whole, it possesses 14 rather than 16 as the diploid complement of chromosomes and appears to justify assignment to a distinct subsection.

Subsection II C. Nuttaliani

C. clavatus S. Wats.—Diploid, 16 chromosomes (fig. 32).

C. kennedyi Porter.—Diploid, 16 chromosomes (fig. 33).

C. aureus S. Wats.—Diploid, 32 chromosomes (fig. 34). Again the various sizes of chromosomes occur in quadruplicate, and this species is tetraploid.



FIGS. 32–38.—Fig. 32, *C. clavatus*, root tip metaphase. Fig. 33, *C. kennedyi*, root tip metaphase. Fig. 34, *C. aureus*, root tip metaphase. Fig. 35, *C. nuttallii*, root tip metaphase. Fig. 36, *C. gunnisoni*, root tip metaphase. Fig. 37, *C. plummerae*, root tip metaphase. Fig. 37*a*, same, metaphase I. Fig. 38, *C. luteus* var. *citrinus* "*vivid*," root tip metaphase, 20 chromosomes. Fig. 38*a*, same, metaphase I. Fig. 38*b*, same, bivalents drawn separately to show complete terminalization of chiasmata at metaphase I.

C. nuttallii Torrey.—Diploid, 16 chromosomes (fig. 35). These four species form a coherent group with their markedly similar karyotypes and similar morphological characters.

Subsection II D. Gunnisoniani

C. gunnisoni S. Wats.—Diploid, 18 chromosomes (fig. 36). This species also requires a subsection of its own because of its cytological and morphological differences from the members of the preceding subsections.

Section III. Cyclobothra

Under section III are included those species with linear unwinged fruits and thick fibrous-reticulate bulb coats.

C. plummerae Greene.—Diploid, 18 chromosomes (fig. 37); 9 pairs at metaphase I of meiosis (fig. 37*a*). Its chromosomes are somewhat thinner than those of any of the other species examined and the karyotype differs in some other respects. It should be interesting to examine other species of the Cyclobothra section for comparison with *C. plummerae* as well as with species of the other two sections. Would they show a similar variation in chromosome numbers and morphology such as is shown in the Mariposa section, or would they possibly have a single basic number throughout, as occurs in the Eucalochortus section?

Doubtful form

A variety secured from Purdy under the name of *C. luteus* var. *citrinus* "*vivid*" showed a diploid number of 20 chromosomes in root tip cells (fig. 38) and 10 pairs at metaphase I of meiosis (fig. 38*a*). There is complete terminalization of chiasmata as shown in figure 38*b*. In this figure the chromosomes, drawn separately, are from a single sporocyte. A similar condition was observed in a number of the sporocytes. This is probably an autotriploid among whose homologous chromosomes translocations and inversions have occurred so as to change their homologies and pairing relations.

Fragmentation

Fragments have been observed in a few of the species and varieties investigated, notably in *C. luteus* var. *citrinus* (fig. 28) and *C. venustus* var. *purpurascens* (fig. 23). A majority of the root tips from

these two varieties have shown them, and they have been seen in the sporocytes also. In some cases a fragment becomes attached laterally to a chromosome (fig. 22), in others terminally to produce a satellited condition (fig. 29). In most instances they remain apparently unattached, although their behavior has not been followed in detail. But neither bulbs, roots, nor plants possessing fragments appear different in any recognizable way from those not possessing them.

Discussion

The Eucalochortus section is much more uniform in practically all respects than is the Mariposa section. All the species examined under the former section show a basic number of 10 chromosomes and relatively little variation in karyotypes, while in the latter section there are species with basic numbers of 6, 7, 8, and 9 chromosomes. Seven is suggested as the original basic number for the Mariposa section and perhaps also for the genus. Species which possess some other number have possibly been derived from one or more of the species in which 14 is the somatic number of chromosomes by doubling the number in one or both types of spores (and gametes) to produce the triploid and tetraploid forms; or as a result of segmentation or translocation, with or without inversions; or through a combination of these processes.

For example, *C. superbus*, with $2n = 12$ chromosomes, may have been derived from *C. luteus*, $2n = 14$ chromosomes, through fusion and translocation between two chromosomes of the latter, one of which, designated as 2 (fig. 40), has a subterminal attachment constriction, and the other, designated as 6, has a submedian one. If during the prophases of a nuclear division in the early development of an embryo, chromosome 2 should become attached just below its attachment constriction to the shorter arm of chromosome 6, at or near its distal end, followed by breakage and detachment of the centromere (kinetic body) with the accompanying small knob of chromosome 2, a new chromosome carrying essentially all the genes of both former chromosomes would result. Since the detached fragment carries few, possibly no, genes, it would probably be eliminated during the division which follows. As a consequence two cells would

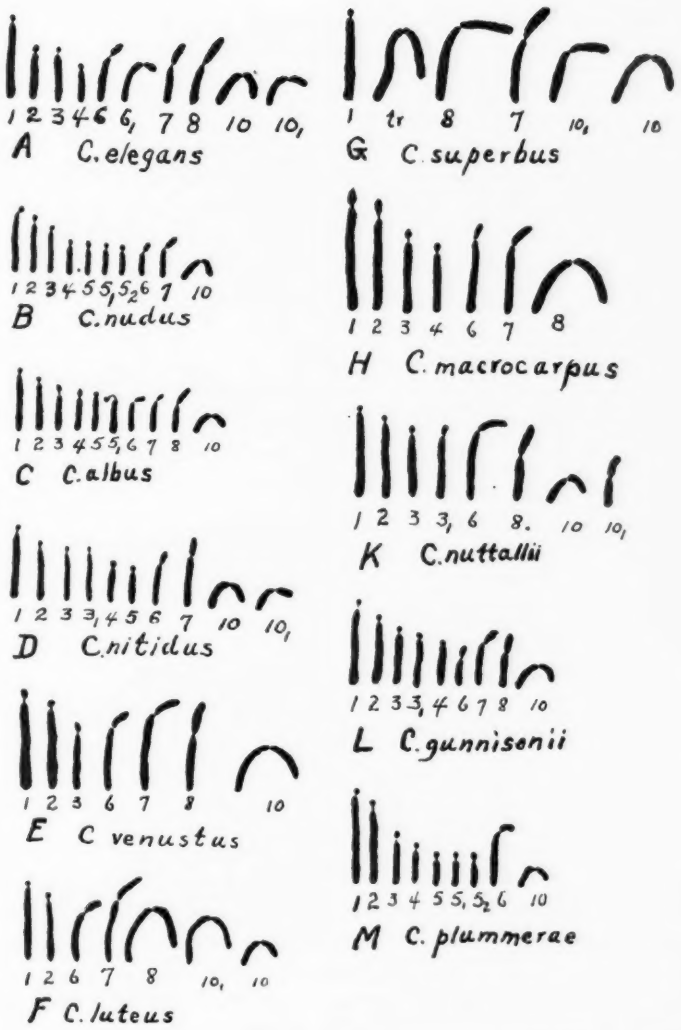

1 2 3 4 6 6, 7 8 10 10,
A C. elegans
1 2 3 4 5 5, 5, 6 7 10
B C. nudus
1 2 3 4 5 5, 6 7 8 10
C C. albus
1 2 3 3, 4 5 6 7 10 10,
D C. nitidus
1 2 3 6 7 8 10
E C venustus
1 2 6 7 8 10, 10
F C. luteus
1 tr 8 7 10, 10
G C. superbus
1 2 3 4 6 7 8
H C. macrocarpus
1 2 3 3, 6 8. 10 10,
K C. nuttallii
1 2 3 3, 4 6 7 8 10
L C. gunnisonii
1 2 3 4 5 5, 5, 6 10
M C. plummerae

FIG. 39.—Haploid chromosome complements selected from representative species of each of the subsections of *Calochortus* and drawn approximately to scale. Numbers indicate the homologies suggested.

result containing 13 instead of the usual 14 chromosomes. From these cells a branch, or branches, might develop and produce flowers containing stamens and pistils. During the prophases of sporogenesis, synapsis would in all probability occur between the translocated chromosome and chromosomes 2 and 6 homologous to it. Two of the four spores resulting from the meiotic divisions would carry the

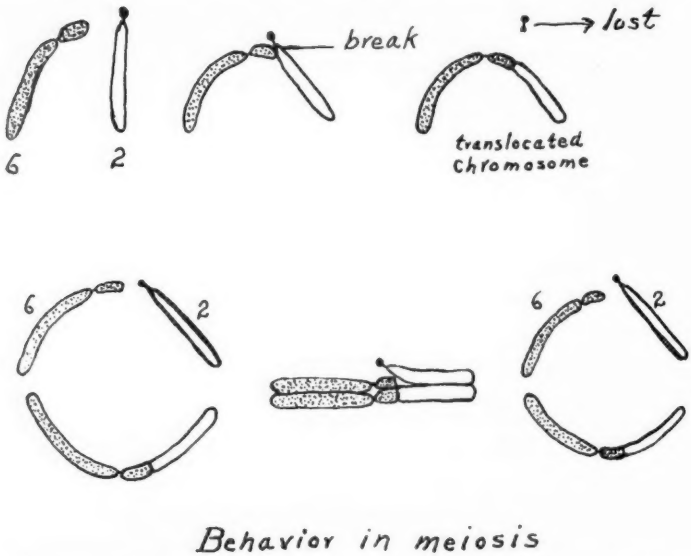


FIG. 40.—Diagram illustrating possible occurrence of fusion and translocation between chromosomes 2 and 6 of *C. luteus* to form the translocated chromosome of *C. superbus*, with accompanying reduction in number of chromosomes.

translocated chromosome, with a total of 6, while the other two spores would contain the 7 unchanged chromosomes. Since only the attachment constriction and the small knob have been eliminated in forming the translocated chromosome, it is probable that the spores containing the new type chromosome would be viable. Union between two gametes, each containing the translocated chromosome, would result in the production of a zygote and subsequently a plant homozygous for the translocation, and with a change in chromosome number from the original 14 to 12. The probable occurrence of such

a change is in essential accord with the dislocation hypothesis of NAVASHIN (4). That this process may actually have occurred is supported by a comparison of the haploid complement of *C. luteus* (fig. 39*F*) with that of *C. superbus* (fig. 39*K*).

An increase in the number of chromosomes may also result from the addition of one or more centromeres combined with appropriate "dislocation," as has been postulated also by NAVASHIN. According to this theory, an extra chromosome is introduced by non-division, non-disjunction, or some other aberration in nuclear division, so that the somatic cells of an individual contain three chromosomes of one kind (a trisome). While all the centromeres remain intact, the materials of one member of the trisome are replaced through elimination and translocation by the materials of one (or more than one) non-homologous chromosome, so that the materials of the latter become associated with two centromeres instead of one. Through disjunction at meiosis the normal homologue of the contributing chromosome is segregated out and gametes with the new chromosomal constitution are formed. Union of two such gametes would result in an individual with an extra pair of chromosomes but with the same assemblage of chromosomal materials. The chromosomes would be able to function normally in both mitosis and meiosis. Thus species with higher numbers might arise from one or more containing 14, and from the evolved ones others with still higher numbers might develop.

Evolution of species with numbers greater than 14 may possibly have occurred also through segmentation of chromosomes whose attachment constrictions are in a median or submedian position, provided the break occurs through the centromere so as to provide functional portions of it to both arms of the segmented chromosome. Both arms might then behave normally in mitosis. Again should this process occur during an early stage in embryogeny, a branch, or branches, might develop flowers containing the fragmented chromosome or chromosomes. During sporogenesis the two fragments would likely pair with the respective homologous arms of the non-segmented chromosome, and as a result of meiotic divisions produce two spores containing an "extra" chromosome and two unchanged spores. Union of two gametes containing the extra chromosomes

would result in an offspring containing two more chromosomes than were present in the parents but with unchanged genetic composition. Subsequent mutations might cause sufficient morphological character changes as to constitute species differentiation, and to effectively prevent cross breeding with the original parental species.

Some evidences for the segmentation hypothesis as presented in the preceding paragraph are based upon a comparison of the chromosome complements as shown in figure 39. For example, should chromosome 7 of *C. luteus* (fig. 39*F*) break through its centromere, it would result in a chromosome configuration similar to that of *C. nuttallii* (fig. 39*K*). Thus a 16 chromosome form might arise from a 14 form. The same type of fragmentation may account also for the higher chromosome numbers, including the 18 and 20 forms. McCLINTOCK (3) has reported one case in *Zea mays* in which the break occurred through the attachment region, so that the two portions of the fragmented chromosome possessed functional parts of it.

Summary

1. The species of *Calochortus* occur naturally only in western North America. A total of twenty-eight species and five varieties representing fourteen species in the Eucalochortus section, thirteen species and five varieties in the Mariposa section, and one species in the Cyclobothra section have been investigated.

2. Somatic numbers of 12, 14, 16, 18, 20, 21, 28, 32, and 40 have been observed, with corresponding gametic numbers of 6, 7, (8), 9, 10, (14), (16), and 20. (Numbers in parentheses have not been observed but are inferred from the somatic numbers.)

3. A rearranged classification, based upon both cytological and morphological criteria, is presented as outlined by MR. MARION OWNBEY. In this classification all species with a basic number of 10 chromosomes and having capsules winged on the angles are placed in the Eucalochortus section, while those species with some basic number other than 10 and having capsules not winged are placed in the Mariposa section, except *C. plummerae*, with n = 9 chromosomes, which for other reasons is placed in the Cyclobothra section. The chromosome numbers and their morphology, together with the

morphological characters of the plants, have made necessary this rearrangement.

4. Seven is suggested as the basic number of chromosomes for the Mariposa section, and perhaps for the genus also. One species, *C. superbus*, with 12 somatic chromosomes, may have been derived from *C. luteus* through fusion and translocation between two chromosomes of the latter.

5. Evolution of species with somatic numbers greater than 14 may have resulted from the addition of one or more centromeres combined with appropriate "dislocation," as postulated by NAVASHIN.

6. It is also possible that fragmentation of medianly or submedianly constricted chromosomes through the centromere so as to give each of the arms a functional portion of the centromere may have resulted in increasing the chromosome numbers, and in the formation of new species.

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DISTRIBUTION AND HYBRIDIZATION OF VERNONIA IN MISSOURI

CORA SHOOP STEYERMARK

(WITH SEVEN FIGURES)

Introduction

This study has been undertaken with the aim of determining, as far as possible, the species of *Vernonia* in Missouri; the hybridizing possibilities of the species; and the extent to which characters persist if pollination is controlled. Three lines of procedure were followed, extending over a period of six years. (1) Dried specimens were studied; (2) plants were studied in their natural habitats; (3) plants were grown from seeds of assumed species and hybrids of different vernonias.

1. More than 1000 dried specimens from seventy-five counties of Missouri were studied and compared in the process of separating species with their variations from other species. Specimens were found which resembled two species so closely that it was a question in which group to place them; these were classed as hybrids. In case of minor variations from the species type, which left no doubt as to where the plant belonged, the specimen was given the species name. It was found that DANIELS' (3) eight new species were hybrids of or variations from the species *V. baldwini*, *V. missurica*, and *V. altissima*.

In the herbarium of the Missouri Botanical Garden several hundred specimens of *Vernonia* from other states in the United States, Mexico, and Central and South America were studied for the purpose of determining the ancestry of our own species; to see what characteristics persisted during migration toward Missouri; and to what extent past structures might have influenced characters of our present species.

2. More than 1000 plants of *Vernonia* were studied in the field in their natural habitat. Special attention was given to altitude, soil moisture, soil type, and sunshine and shaded areas to determine if

possible what bearing these had upon the species, and to what extent they caused variations.

3. Seeds from hybrids, and from plants which because of radically distinct characteristics and wide distribution would seem to be new species, were planted in garden plots and observed for from one to three generations.

From this study it is concluded that, for Missouri, only the five species described in the key are distinct. *V. interior* does not appear to be distinct enough to justify inclusion as a species; it needs more genetical and cytological investigation.

Key to Missouri species of Vernonia Schreb.

Heads large, 8–16 mm. high and 10–18 mm. broad; number of achenes 60–115; involucral bracts tipped with long filiform appendages; leaves chiefly glabrate above and below.... *V. crinita*

Heads small, 4–8 mm. high and 5–9 mm. broad; achenes 14–60.

Leaves glabrate or with pubescence on veins of lower surface.

Involucral bracts appressed, chiefly acute.

Inflorescence a fastigiate cyme; leaves deeply pitted[1] beneath..... *V. fasciculata*

Inflorescence a panicle; leaves not pitted..... *V. altissima*

Leaves from pubescent to tomentose on lower surface.

Involucral bracts acute, recurved, resinous on each side of prominent midvein.

Bracts broadly acute or narrowly acuminate, abruptly or only slightly recurved..... *V. baldwini*

Involucral bracts chiefly obtuse, appressed, midvein not prominent..... *V. missurica*

Observations

Ecological variation and distribution

According to Gleason (4) there is no correlation between structure and habitat. Field observations indicate in a general way that the typical xerophytic *Vernonia* grown in the sun has thicker and more tomentose leaves.

[1] Dot-like depressions.

Individual plants under observation for six consecutive years in the garden plot showed from year to year considerable variation in height of plant, length and width of leaves, color of stalk, leaves, and pappus (table 1). These variations were due to the only variant factors—rainfall, number of days of sunshine, and possibly temperature.

YAPP (12) regarded the action of light and water as a factor in determining the structure of "sun" and "shade" leaves. He found

TABLE 1

VARIATION BETWEEN PARENT PLANT, V. CRINITA, AND SEEDLING F_1 AND VARIATIONS OF F_1 IN SUCCEEDING YEARS

V. CRINITA	HEIGHT (FEET)	INVOLUCRE		LEAVES		SCALES	CHARACTER OF STEM AND LEAF
		HEIGHT (CM.)	WIDTH (CM.)	WIDTH (CM.)	LENGTH (CM.)		
1884 P_1...	3	0.6	1.4	1–1.4	12–14	Cauline, not exceeding height of involucre	Smooth and purple tinted
1884 F_1							
1935....	5½	1.2	2	2	12–15	Scale appendages of typical V. crinita long and curling, much exceeding height of involucre	Smooth and purple tinted
1936....	5½	1.1	2	0.5–0.7	10–13		
1937....	6	1.4	2.1	0.8–1.5	11–13		

that the degree of pubescence followed closely the mean curve of evaporation and light intensity in both space and time. MAXIMOV (6) stated that "a decrease of soil moisture induces changes in leaf structure in the direction of increased xeromorphy." He also found that in plants growing in deep shade the upper and lower leaves show less difference in venation and pubescence than when growing in sunshine. POOL (9) stated that the xerophytic leaf is thick and the stoma-bearing surface hairy.

V. crinita, and *V. crinita*×*V. missurica* of the Schwieder habitat, 8 miles east of Steelville, Missouri, had decided tomentose lower leaf surface, stems, and bracts. These plants, which grew along a spring

branch, were rooted in swamp much of the summer, yet exposed to sunshine all day. Seeds taken and grown in the garden plot produced typical *V. crinita.*

The general order of ecological distribution of the common species found in the Ozarks, beginning with lowlands and ascending in altitude, is: *V. crinita*, *V. altissima*, *V. missurica*, and *V. baldwini.*

V. crinita was collected from forty-four counties south of the Missouri River. It is definitely confined to the lowlands of the Ozarks. PALMER and STEYERMARK (**8**) found that *V. fasciculata* occurred on prairies, meadows, and alluvial soils along streams of twenty-eight counties, chiefly in northern and central Missouri. *V. altissima* also occurred in lowlands in twenty-eight counties, chiefly in eastern, central, and southern Missouri. *V. missurica* was found distributed generally in forty-four counties, occurring very commonly in the northeastern part of the state. *V. baldwini*, collected in fifty-four counties, apparently has the widest distribution. It is least commonly found in northwestern Missouri. Herbarium specimens labeled *V. interior* were examined from twenty-five counties.

Of interest is the absence of all vernonias above a certain elevation in Dent and Shannon counties along highway 19. The upland soil along most of this highway is Clarksville stony loam, which dries very quickly. From about 5 miles north of Salem to 10 miles south, the upland soil is of Hanceville loam. This soil is loose, thin, and of low fertility. Forests and much vegetation common to lower levels were growing to the summit of the highest points, but *Vernonia* ended at definite levels on either slope of the hills, owing probably to a lack of soil moisture.

The H ion concentration of the soil may have some influence in the distribution of *Vernonia;* however, only one test was made. Several samples of soil were tested from different parts of a 4-acre field 2 miles east of Sigo in Dent County, where *V. crinita* was the only species growing. The test showed no trace of acidity. The reaction ranged from neutral to slightly alkaline. KURZ (**5**) found a correlation between H ions and the distribution of a number of species, but he examined no *Vernonia.*

One colony of white *V. altissima albiflora* Raf. was found 10 miles west of Steelville near the Meramec River. The colony consisted of

twelve separate clumps, in some places 15 feet or more apart. The flowers are pure white, pappus tan or golden, stems, leaves, and bracts pale green and consistently smooth. Also one white-flowered plant of the same species was found 2 miles east of Columbia, Missouri, in a highland forest, in August, 1932. It had the characteristic smoothness, while all other plants of *Vernonia* growing near were very pubescent forms of *V. altissima*.

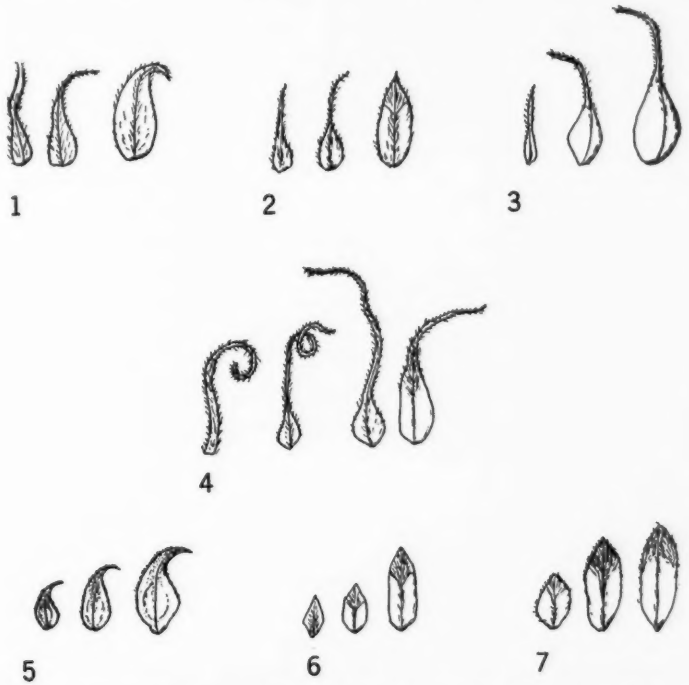
BUSH (2) made a study of the mound flora of Atchison County during August, 1895. He reported seventy-nine species, but did not mention *Vernonia* for that county.

EFFECTS OF NATURAL GROWING

Uniformity of habitat, including climatic, edaphic, and biotic factors, tends to produce plant communities, according to NICHOLS (7), in which species within a certain genus take on similar characteristics. TURESSON (11) stated, "Similarity in growth form shown by different species when inhabiting similar habitats is merely a modification induced by similar habitat factors, while the similarity in growth form of a plant inhabiting very extreme habitats is held to be of hereditary nature." The similarity of different species of *Vernonia* in each of the habitats mentioned for Missouri is to a greater extent due to crossing than to edaphic factors. Some examples illustrate this position. A colony of *Vernonia* in southern Howell County, considered a hybrid between *V. baldwini* and *V. crinita* (fig. 1), had most of the characteristics of typical *V. baldwini* (fig. 5), but possessed the long, curling, filiform scale appendages of typical *V. crinita* (fig. 4). These plants were growing on a high prairie, a typical *V. baldwini* habitat. *V. crinita* was growing several miles away along creeks, and *V. baldwini* was found among the assumed hybrids. *V. baldwini*×*crinita* of figure 1 had the general appearance of *V. baldwini* at a distance, with an involucre height of 5 mm. and a width of 8 mm. The leaves were 3 cm. wide by 10 cm. long, obtuse at the base, with lower leaf surface very pubescent. Other similar hybrids of *V. baldwini* and *V. crinita* were found 8 miles east of Steelville, 5 miles west of Steelville, at Steelville, and in Texas County. In no place north of the *V. crinita* region was any plant found

bearing *V. crinita* characteristics. Figure 2 shows a cross of *V. crinita* with *V. altissima*. Figure 6 shows bracts of typical *V. altissima*.

V. baldwini and *V. altissima*, with fastigiate cymes and leaves and bracts closely resembling *V. fasciculata*, were found frequently in



FIGS. 1–7.—Involucral scales, from smaller to larger: Fig. 1, *V. baldwini*×*V. crinita* from Howell County; fig. 2, *V. altissima*×*V. crinita* from 2 miles east of Sligo; fig. 3, *V. crinita*×*V. missurica* from 8 miles east of Steelville; fig. 4, *V. crinita* herbarium specimen no. 1056; fig. 5, *V. baldwini* no. 1120; fig. 6, *V. altissima* no. 1004; fig. 7, *V. missurica* no. 1138.

the northern part of the state. Twenty-five specimens examined from Buchanan and Andrew counties showed hybridization between *V. fasciculata* and *V. baldwini*.

Where *V. missurica* is the dominant species, there is a tendency for the other species of *Vernonia* to hybridize with it.

In the Ozarks, plants of every other species of *Vernonia* common to the state, except *V. fasciculata* which does not occur in that region, were found with resemblance to *V. crinita* in either or both of the following: a marked increase in length of scale appendages and increased size of head; or in type of leaf structure. This gives evidence that *V. crinita* has crossed with the other species, producing wide variations. The typical *V. crinita* follows the alluvial and gravel soils of river and creek bottoms at an altitude between 500 and 750 feet. Evident crosses of *V. crinita* with *V. missurica* are shown by bract variations (fig. 3). Both involucre and leaves tend toward a median between the species crossed. Figure 7 shows bracts of typical *V. missurica*.

TABLE 2

AVERAGE NUMBER OF ACHENES PER HEAD IN TYPES AND HYBRIDS OF VERNONIA

VERNONIA	V. FASCICULATA	V. ALTISSIMA	V. INTERIOR	V. BALDWINI	V. MISSURICA	V. CRINITA
fasciculata	(20)*	.....	.....	.....	.....	.....
altissima	.....	(22)	.....	.....	.....	52
interior	28	.....	(28.8)	.....	29.5	.....
baldwini	.....	.....	.....	(29)	.....	54
missurica	.....	.....	36	.....	(41)	49
crinita	.....	.....	.....	48	52	(85)

* Types given in brackets.

Many species and hybrids were examined to obtain the average number of achenes per head in an attempt to determine what relationship existed between the two supposed parents and the hybrid. Table 2 shows the results obtained.

GROWTH FROM SEEDS

On October 3, 1931, twenty-five plots in the garden were planted with seeds from twenty-five specimens of *Vernonia*, including types and variations of *V. crinita*, *V. baldwini*, *V. interior*, *V. altissima*, and *V. missurica*. Sixteen pots were planted in the house November 1, 1931. Of these sixteen, half the seeds were treated for 2 hours with concentrated sulphuric acid and half were left untreated. On November 28 of the same year, twelve plants appeared in treated pot no. 1026, *V. crinita*. About the same number of plants appeared

in untreated pot no. 1027, *V. crinita*. March 17, 1932, five seeds germinated in treated pot no. 1035, *V. altissima*. April 10, one seed germinated in treated pot no. 1037, *V. baldwini* (DANIELS' new species, *V. flavipapposa*, with the golden pappus). May 2, one plant appeared in outdoor plot no. 1007, *V. crinita*, and also five seeds germinated in outdoor plot no. 1012, *V. baldwini* (*V. flavipapposa* Daniels). June 1, one plant appeared in treated pot no. 1029, *V. baldwini*, oval-leaved form.

Seeds were used from plants collected in the field without any attempt to prevent crossing. Most of the fruits which failed to germinate were collected in August and probably were not mature. May 4, 1932, the potted plants were transplanted to the garden.

Of the seven plants raised from the one head of no. 1026, *V. crinita*, six seedlings closely resembled the parent plant. Individual no. 5 F_1 of no. 1026 P_1 showed an evident cross with *V. altissima*. The involucre of the parent *V. crinita* measured 8 mm. high and 14 mm. wide with filiform appendages more than equaling the height, while the involucre of no. 5 F_1 measured 6 mm. high and 7 mm. wide, with slender, straight, erect appendages barely reaching the rim of the involucre. The leaves of the seedling exceeded the parent in both length and width (table 3); otherwise there was close resemblance. *V. altissima* was growing on the plot from which no. 1026 P_1 was taken.

The scale appendages of individual no. 7 F_1 of no. 1026 P_1 were longer and more curled than those of the parent, completely enveloping the head until in full blossom.

V. baldwini, no. 1037 P_1, with golden colored pappus and lanceolate, tapering leaves, produced an F_1 with tawny pappus, showing decided purple in parts not exposed and blunt ovate leaves 4.5×9.5 cm., illustrating another cross in the field. An oval-leaved *V. baldwini* was growing in the plot where no. 1037 P_1 was collected.

The one plant from seeds of *V. baldwini*, no. 1012 P_1, described by DANIELS as *V. flavipapposa*, resembled the parent in every respect the first year; however, the pappus color varied from year to year. In 1933 the color was a dull purple near the base, becoming tawny with age. In 1936 the color took on the glossy golden shade of the parent. In 1937 the pappus on each of the forty-seven stalks from

TABLE 3

VARIATION BETWEEN PARENT PLANT AND SEEDLING FLOWER IN EACH SPECIES, GROWN SEPTEMBER 10, 1932

VERNONIA		ACHENES	PAPPUS	HEADS	INVOLUCRE (MM.)	SCALES	STEM	LEAVES
baldwini 1037	P_1	35	Golden	130	height 5 width 6	Typical	Green	Lanceolate 2.5×9.5 cm.
	F_1	30	Tawny	90	height 5 width 6	Typical	Green	Ovate, blunt 4.5×9.5 cm.
baldwini 1012	P_1	25	Golden	125	height 5 width 6	Typical	Green	Lanceolate 1.7–2×4–10 cm.
	F_1	25	Golden	72	height 5 width 6	Typical	Green	Lanceolate 2–3.5×3–10 cm.
crinita 1007	P_1	85	Purple	29	height 9 width 16	Filiform	Green	Finely serrated 1×10 cm.
	F_1	84	Purple	63	height 9 width 16	Filiform	Green	Coarsely serrated 2.5×14 cm.
crinita 1026	P_1	105	Tawny	50	height 8 width 14	Filiform	Red	1.3×14 cm.
	(1) F_1	80	Tawny	21	height 7 width 14	Filiform	Green	1.7×10 cm.
	(2) F_1	?	Tawny	25	height 7 width 14	Filiform	Green	2–3×16 cm.
	(3) F_1	?	Tawny	15	height 7 width 14	Filiform	Green	2–3×16 cm.
	(4) F_1	?	Tawny	13	height 7 width 14	Filiform	Green	2–3×16 cm.
	(5) F_1	?	Tawny	26	height 6 width 7	Erect Exceeding the head	Red	2.5×16 cm.
	(6) F_1	?	Tawny	16	height 8 width 14	Longer, more prominently curled than P_1	Green	1.7×14 cm.
	(7) F_1	?	Tawny	38	height 8 width 14	Filiform	Green	1.5×15 cm.
altissima 1031	P_1	26	Tawny	500	height 5 width 6	Typical	Red	Smooth 2–3×15 cm.
	(1) F_1	?	Tawny	85	height 5 width 6	Typical	Red	Puberulent
	(2) F_1	?	Tawny	92	height 5 width 6	Typical	Red	Smooth 2×12 cm.
	(3) F_1	?	Tawny	62	height 5 width 6	Typical	Red	2×13 cm.

the original rhizome was a dull straw color. As seen through the low power of a compound microscope, the bristles appear barbed, with varying numbers of erect, more or less appressed barbs. The purple pappus is more densely barbed than the golden. The pappus of this parent plant is the glossy golden color which caused DANIELS to separate it as a distinct species. No. 1012 P_1 was taken from 2 miles east of Sligo, the only individual with this type of pappus within a quarter of a mile of the plot. *V. baldwini*, with this conspicuous and attractive golden pappus when dry, is found in the approximate ratio of 1 plant to 500 of the typical *V. baldwini*. Of the specimens observed in the herbarium of the Missouri Botanical Garden, the same type of pappus was found in one specimen of *V. altissima*, one of *V. noveboracensis*, and one of *V. glauca* from other states.

Herbarium specimens of the ancestors of our species from South America, Central America, Mexico, and the West Indies which were examined in the Missouri Botanical Garden showed very little variation in pappus color, ranging from pale cream to golden. Only two exceptions were noted: one specimen from Brazil had a nearly black pappus and another from the same country had a tawny pappus. It seems that the purple pappus is characteristic only of species in the United States. The occasional occurrence of the golden pappus here might indicate that it is a reversion to the color of the ancestral species of South America, pollen from one plant heterozygous for that color having fertilized the megagamete of another plant also heterozygous for the same ancestral color of pappus. Another possibility exists, that of mutation. In either case DANIELS' *V. flavipapposa* certainly crosses with such ease that it does not perpetuate itself as a species.

V. baldwini, no. 1012 F_1, showed also some leaf variation in size in succeeding years. On the parent plant, no. 1012 P_1, leaf measurements halfway down the stem were 1.7–2×4–10 cm. On F_1 they were: in 1932, 2–3.5×3–10 cm.; 1933, 4–5×12 cm.; 1936, 4–5×9 cm.; 1937, 5–5.5×14–15 cm.

Individual F_1 of parent 1029, *V. baldwini*, with oval leaves, resembled the parent closely in leaf form. However, it failed to live to maturity.

Since the seeds of no. 1026 P_1, *V. crinita* (table 3), all came from

the same head, and since the seven seedlings of this parent had practically the same environment, the resulting variations must be genetic.

In September, 1934, seeds were collected from specimen *V. crinita*, no. 1884, with short cauline appendages and narrow leaves. These were planted in November of the same year in the garden plot. The parent plant, growing at the base of a west-facing, rocky hill, was only 3 feet high. Other typical *V. crinita* were growing along the creek bed 10 feet away. All fourteen plants of the F_1 generation, with their large heads and long curling scale appendages, showed very little similarity to the small head and short acute appendages of the parent plant. It will be noted in table 1 that while the height of F_1 and the size of its involucre nearly doubled that of P_1, the leaves of F_1 were narrower. Hence in every respect the F_1 tended to approach the typical *V. crinita*.

May 3, 1934, sixty-five plants appeared from seeds gathered October 19, 1933, from a colony of *V. altissima albiflora*, no. 1883, and were planted in the garden November 1, 1933. Detailed records were kept for sixteen of these progeny. Out of the sixty-five seedlings, fourteen resembled the parent in every respect, with pure white flowers, smooth yellow-green leaves, stems, and heads. These plants were also very consistent in leaf size, shape, and serrations. The shape of the head varies somewhat from *V. altissima* in being slightly more elongated and more pointed. *V. altissima albiflora* can readily be distinguished from other *V. altissima* in the garden plot before bud color begins to show. Twenty-six other individuals of the F_1 had green heads, leaves, and stems, with purple showing only in the notches of the leaves. The remaining twenty-five were definitely purple tinted in all parts (table 4). In the purple forms there was wide variation in pubescence from smooth to heavily pubescent. Leaves varied from 10 to 25 cm. in length and from 2 to 5 cm. in width. Pappus color varied from tan to purple.

Heads of white-flowered individual no. 4 F_1 of no. 1883 P_1 were covered with waxed bags during flowering to prevent cross pollination. Seeds from these heads were planted November 20, 1934, in the garden plot. Of the four seeds which germinated, two of the F_2 were typical of the white-flowered parent and two were light green

TABLE 4

CHARACTERISTICS OF F_1 AND F_2 OF V. ALTISSIMA ALBIFLORA 1883 P_1

V. altissima albiflora	Color of flower	Color of leaf, stem, and bracts	Leaves		Character of leaves, stem
			Width (cm.)	Length (cm.)	
1883 P_1	White	Light green	1.5–2	10–14	Smooth throughout
1883 F_1 No. 4	White	Light green	2.5	10–12	Smooth throughout
1883 F_2 No. 17	White	Light green	2–2.1	10–13	Smooth throughout
1883 P_1	White	Light green	1.5–2	10–14	Smooth throughout
1883 F_1 No. 10 1935	Purple	Dark green with much purple	3–4	17–20	Very pubescent
1937	Purple	Dark green with much purple	3–5.5	18–25	Very pubescent
1883 F_2 No. 20	White	Yellowish green	2–3	12–15	Smooth throughout
No. 21	Purple	Dark green with much purple	2.5–3.5	16–18	Pubescent
1883 P_1	White	Light green	1.5–2	10–14	Smooth throughout
1883 F_1 No. 11	Purple	Light green with purple only in leaf notches	3–3.5	18–20	Smooth leaves
1883 F_2 No. 19	Purple	Light green with purple only in leaf notches	4.5–6	18–24	Pubescent
1883 F_1 No. 1	White	Light green	2.5–4	12–15	Smooth throughout
1883 F_1 No. 2	Purple	Green with much purple	2.3–3	15–18	Smooth
1883 F_1 No. 3	Purple	Dark green with much purple	3–5	20–25	Very pubescent
1883 F_1 No. 6	Purple	Purple in notches of leaves	2.5–3	10	Pubescent
1883 F_1 No. 7	Purple	Light green	2.5–4	15–19	Very pubescent
1883 F_1 No. 12	Purple	Dark purple	1.5–2	12–14	Slightly pubescent
1883 F_1 No. 13	Purple	Dark purple	2–3	12–15	Smooth
1883 F_1 No. 15	White	Light green	2	12–15	Smooth
1883 F_1 No. 16	Purple	Dark purple	2–4	17–22	.....

in coloring throughout, with purple in leaf notches and purple flowers. Likewise the heads of purple-flowered individual no. 10 F_1 of no. 1883 P_1 were covered. Seeds from these produced an F_2 generation of two typical white and fourteen dark purple individuals.

Seeds from covered individual no. 11 F_1, with light coloring, produced eight F_2 individuals very similar to the parent.

The common occurrence of *V. crinita*×*V. missurica* (fig. 3) with rather constant characteristics, found over widespread areas where *V. crinita* and *V. missurica* were present, would seem to warrant its description as a species. Experiments in growing succeeding generations from this hybrid, however, prove that the characters which set it aside as new are not transmissible.

While species grown in pots by BOHN (1) failed to flower the same year the seeds germinated, the seedlings listed in this report flowered from four to six months after germination.

Summary and conclusions

1. More than 1000 dried specimens of species of *Vernonia* from seventy-five counties of the state of Missouri were studied and compared in the process of separating species with their variations from other species and in disposing of hybrids. It was found that DANIELS' (3) eight new species were hybrids or variations from the species *V. baldwini*, *V. missurica*, and *V. altissima*.

2. In the herbarium of the Missouri Botanical Garden several hundred specimens of *Vernonia* from other states in the United States, Mexico, and Central and South America were studied for the purpose of determining to what extent past structures might have influenced characters of our present species.

3. On the basis of field characteristics, *V. interior* did not appear separable from *V. baldwini*. Recent investigations have led to its classification as *V. baldwini* var. *interior* Schubert (10).

4. More than 1000 plants of *Vernonia* were studied in the field in their natural habitats. Special attention was given to altitude, soil moisture, soil type, and sunshine and shaded areas, to determine if possible what bearing these had upon the species, and to what extent they caused variations. It was found that minor characteristics such as size, texture, and pubescence of leaf, pubescence of stem,

number of heads, or pubescence of bracts, are influenced to some extent by ecological conditions; but these characteristics are not transmissible.

5. Some species of *Vernonia* are confined to specific regions in the state.

6. The experiments in growing the species led to four conclusions:

(a) Species bloomed the same year they germinated in outdoor plots.

(b) Some plants with wide distribution which looked like new species in the F_1 generation appeared as the typical species which the parent most closely resembled, indicating that the variation was brought about in the first place (1) by environment after the seed had germinated; (2) by somatic modifications brought about by the environment; (3) by a chromosomal variation caused by environment; or (4) by crossing in the field, but not transmissible. Such experiments show that field characteristics alone are not always a safe guide in naming a new species.

(c) Seeds from parents true to type produced some individuals true to type and some plants that indicated crossing in the field.

(d) *V. altissima albiflora*, if field crossing with *V. altissima* is controlled, will produce the usual genetic ratio of pure *V. altissima albiflora*.

7. The results of the three lines of investigation have led to the conclusion that five distinct species of *Vernonia* are rather constant for the state: *V. crinita*, *V. baldwini*, *V. altissima*, *V. missurica*, and *V. fasciculata*, which vary widely. Any one of these five species apparently may cross with each of the other four, producing a wide variety of hybrids.

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ATAVISTIC LEAF FORMS OF VARIOUS SPECIES OF TREES

ELIZABETH S. OLIVER

(WITH TWENTY-THREE FIGURES)

Introduction

Studies of atavistic leaves have been made by VON ETTINGSHAUSEN and KRAŠAN (2) and by NOÉ (19). The writer plans to carry the work further, making a study of various additional genera.

It is generally known that if the leaves of trees or shrubs are destroyed, after a time new leaves appear which do not exhibit the characteristics of the normal leaf. In like manner leaves of adventitious shoots and very young sprouts exhibit atypical leaves. It is believed that such leaves represent a reversion to an ancestral type. With this in mind, leaves of *Populus tremuloides* Michx., *Corylus americana* Walt., *Tilia americana* Linn., *Ulmus americana* Linn., and *Platanus occidentalis* Linn., were collected and studied. In each case the normal leaf form was determined and the amount of variation typical to the normal leaf noted. Then atypical leaves were studied and compared with fossil species, Cretaceous through Tertiary in age. Pleistocene species were felt to be too recent for such comparative use.

Observations

POPULUS TREMULOIDES MICHX.

Considerable variation in leaf form was found among the normal leaves of this species. After a survey of such variation, leaves believed to be atavistic were studied. These were collected from three sources: (1) shoots which had sprung up after the tree had been cut down; (2) young sprouts; and (3) those resulting from artificial conditions; that is, the first, normal leaves were destroyed the latter part of May and the new leaves which resulted in July were collected for study. All these leaves were found to be atypical and therefore are believed to be atavistic. Comparison was then made with fossil species of *Populus* from the Tertiary and Cretaceous of North

America. Many fossil species were too fragmentary to attempt comparison but showed great similarity to the living atypical leaves. Only fossil leaves which are believed to be duplicated by living ones are included in the following list. Seven fossil species, all Tertiary in age, were found represented in the atypical leaves of the one living species, *P. tremuloides*.

Populus arctica Heer (10)

KNOWLTON (3) states that the species is greatly in need of critical revision. The species was studied as described in the literature, with no attempt at revision. It has a great variety of forms, having margins undulate to dentate to entire. No leaves of *Populus tremuloides* were found having entire margins, but some very nearly approximated those fossils figured with dentate margins. No living specimen has so cuneate a base as the fossil species, but aside from this point some living atavistic leaves resemble the fossil in every detail.

Populus amblyrhyncha Ward (21)

The distinctive character of the leaf of this species is its long blunt apical lobe and the fact that the lateral nerves curve and unite with the midrib. These characters are represented in several leaves of *Populus tremuloides* collected from adventitious shoots formed when a small tree was cut down. The living species differ in one feature: the innermost laterals do not unite with the midrib above the base of the leaf. It is believed that the leaf figured by WARD (24) might show the character of the living specimen referred to if the base of the specimen were complete.

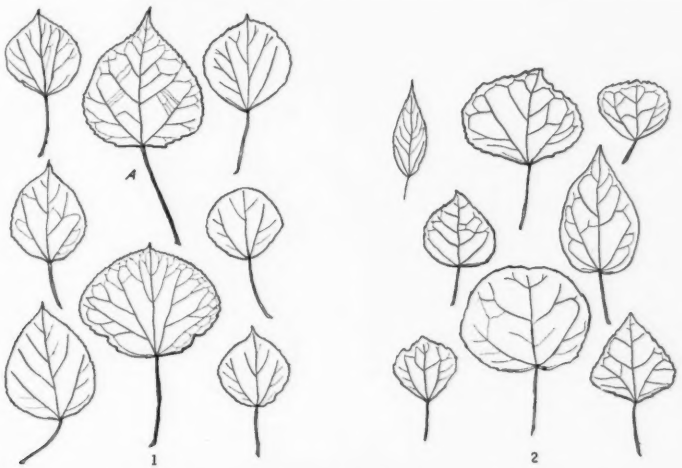
Populus cuneata Newb. (16, 18, 23)

The leaf of *Populus cuneata* is wedge-shaped, obovate, has an obtusely pointed apex, is irregularly dentate, and has three main veins which terminate in the apical portion. The living leaf referred to this species is one which grew on the tip of a branch after the first leaves had been destroyed. On the living leaf the two main secondaries originate above the base, differing at this point from the representative *P. cuneata* with which it is compared. However, other representative leaves of *P. cuneata* have the character represented in the *P. tremuloides* leaf. Also the apex of the living leaf is eaten off,

but the apexes of other leaves on the tip of the same shoot are blunt, greatly resembling the characteristic *P. cuneata* apex.

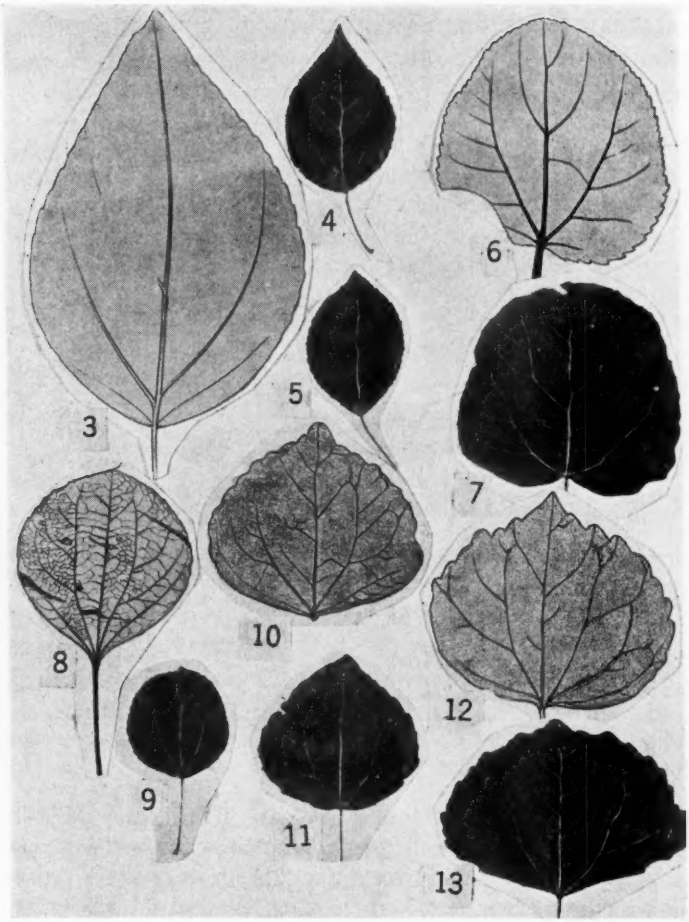
Populus eotremuloides Kn. (**4, 6**)

KNOWLTON has already referred leaves of this fossil species to those of the living *Populus tremuloides*, finding their equivalent in leaves of a young seedling collected in Utah. One small difference



FIGS. 1, 2.—Fig. 1, typical leaf (*A*) of *Populus tremuloides* and normal variations. Fig. 2, atavistic leaf forms of same species.

was noted, namely, that the leaf margin of the living species is toothed from the apex to the base while that of the fossil species is entire at the base. KNOWLTON states, "The leaves of this seedling differ somewhat from the normal mature leaves of the species, but it would seem, in view of their evident relationship to the fossil leaves, that they represent a reversion to an ancestral type, or perhaps rather a survival of this type, which disappears in the mature plant" (**4**). It is believed, therefore, that these seedling leaves represent atavism. In like manner the fossil leaves greatly resemble the majority of second leaves formed on mature trees of *P. tremuloides*. These leaves are also believed to be atavistic. The first leaves were



FIGS. 3–13.—Fig. 3, *Populus eotremuloides*. Figs. 4, 5, *P. tremuloides*, second leaves; Frankfort, Mich. (*cf. P. oetremuloides*). Fig. 6, *P. lindgreni*. Fig. 7, *P. tremuloides;* leaves from adventitious shoots; Lake Ann, Mich. (*cf. P. lindgreni*). Fig. 8, *P. arctica*. Fig. 9, second leaf of *P. tremuloides;* Frankfort, Mich. (*cf. P. arctica*). Fig. 10, *P. amblyrhyncha*. Fig. 11, *P. tremuloides* from adventitious shoot; original tree cut down; Lake Ann, Mich. (cf. *P. amblyrhyncha*). Fig. 12, *P. oxyrhyncha*. Fig. 13, *P. tremuloides*, collected from young shoot; Frankfort, Mich. (*cf. P. oxyrhyncha*).

severed from the trees about May 28 and the new leaves which appeared have the characters of *P. eotremuloides*. They are very much smaller than those of the fossil species, owing to drought, but the main characteristics of the fossil species are well represented.

Populus lindgreni Kn. (**5, 7**)

A great number of leaves approximate this species, but no one of them has all of the characters. Several leaves, however, all from adventitious shoots formed as a result of cutting of the main tree, show all the major characters. One leaf exhibits the circular shape of *Populus lindgreni* and the correct veining and margin, except that the angle between the main secondaries and the midrib is too great. Another exhibits the correct angle between the main secondary but not the correct shape. The margin of the fossil species is toothed, while on all individuals of the living species referred to *P. lindgreni* the base of the leaf is entire. This is the only constant difference between the fossil species and those living leaves referable to it.

Populus oxyrhyncha Ward (**22, 26**)

One leaf taken from a young shoot was found to have most of the characters of *Populus oxyrhyncha*. The apex is not so acute as that in the figured specimens, and the inner lateral primaries do not curve inward toward the apex as much as do those in the fossil species. Aside from these two points the living leaf fits the description of *P. oxyrhyncha*.

Populus neotremuloides Kn. (**9**)

The one figured specimen of *Populus neotremuloides* is very fragmentary. KNOWLTON referred it to the young vigorous sprouts of *P. tremuloides*, finding the characters of the fossil well represented in this living species. Here the fossil is also compared with a large leaf from a young sprout. It does vary from the fossil description, which says the margin is entire at the base and slightly toothed to undulate on the upper part of the leaf. The margin of the living specimen is very coarsely and irregularly toothed throughout. Also the second pair of main secondaries curves downward to merge with the midrib, and the general venation is not so coarse.

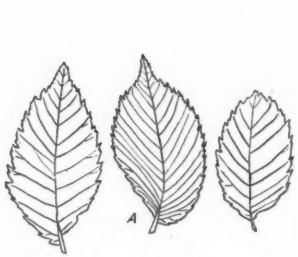
The fragmentary nature of the fossil leaves some doubt as to its

shape and the true nature of the margin. The venation of the fossil and the living leaves is very similar, KNOWLTON having already noted this similarity, referring his species to a leaf of this living species also from a young sprout.

Age of fossil species represented by atavistic forms of the living *Populus tremuloides:*

P. arctica	Eocene, Oligocene
P. amblyrhyncha	Eocene
P. cuneata	Eocene, Oligocene, Miocene (?)
P. eotremuloides	Eocene
P. lindgreni	Eocene, Miocene
P. neotremuloides	Eocene
P. oxyrhyncha	Eocene

It may be noted that the preponderance of fossil species to which the atavistic forms of *Populus tremuloides* may be referred occurs in the Eocene. Since there is such a wide variation in the normal leaf



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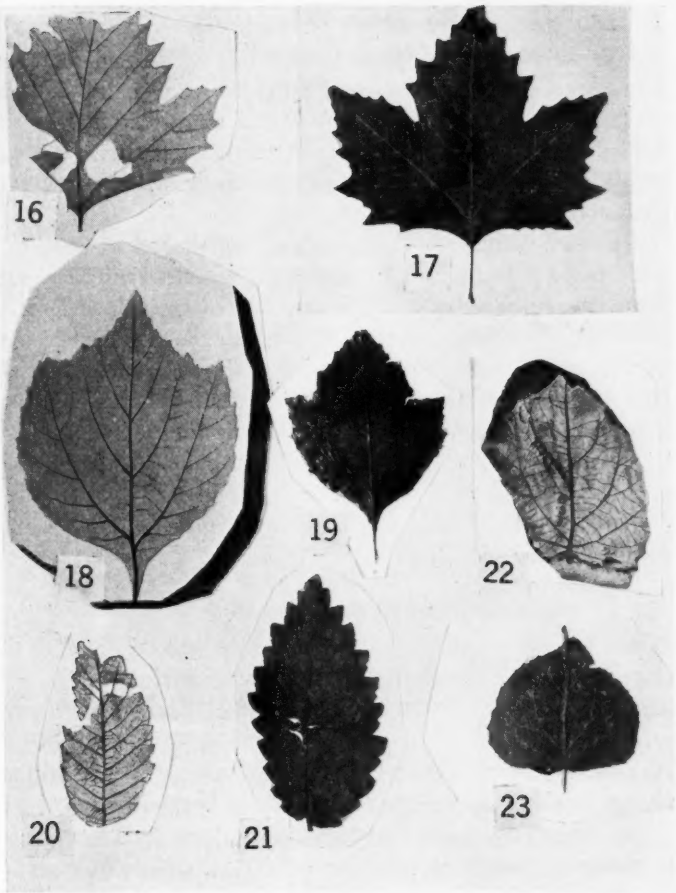
FIGS. 14, 15.—Fig. 14, typical leaf (*A*) of *Ulmus americana*, and leaf variation on normal shoot. Fig. 15, atavistic leaves of same species.

form of the present *P. tremuloides*, there might be a possibility that the present species is somewhat unstable as to leaf form and thus the atavistic forms revert back farther, representing fossils found in the earliest Tertiary.



FIGS. 16–23.—Fig. 16, *Platanus latior grandidentata*. Fig. 17, *P. occidentalis;* Carleton College nursery, Northfield, Minn. (*cf. P. latior grandidentata*). Fig. 18, *P. raynoldsii*. Fig. 19, *P. occidentalis;* Carleton College Nursery (*cf. P. raynoldsii*). Fig. 20, *Ulmus wardii*. Fig. 21, *U. americana;* leaf from adventitious shoot from base of stump after tree had been cut down; Nerstrand, Minn. (*cf. U. wardii*). Fig. 22, *P. neotremuloides*. Fig. 23, *P. tremuloides;* leaf from young sprout; Lake Ann, Mich. (*cf. P. neotremuloides*).

CORYLUS AMERICANA WALT.

Great variation in leaf form is exhibited in the living species. Leaves which have been damaged by grazing are coarse and very pubescent. Those unhurt seem to be much thinner, less pubescent, and less finely serrate. The veins of the normal leaves are primarily alternate. As the leaves show atavism there is a tendency for veins to become opposite.

Corylus americana, the fossil species, seems to be made up of a great variety of leaf form and venation. Some representatives (**27**) correspond to the typical leaf of the modern species, while other representatives of the same species, all from the Fort Union flora, are represented in the atavistic types. The normal leaves of the living species characteristically have alternate primary venation, while the atavistic leaves have a tendency toward the primary veins becoming opposite. The primary veins of the fossil species are generally alternate.

All of the variation of leaf characteristics in the fossil leaf species is represented in leaves of the living species.

TILIA AMERICANA LINN.

There is much variation in the form of the normal leaves in the living species. They are five, six, or seven nerved; the base is asymmetrical, deeply cordate to flat, and the apex is acute to acuminate.

Leaves developing as the result of defoliation or from adventitious shoots after the main tree has been cut down are generally larger in size, but there is so much variation among the normal leaves that the increase in size is their major distinction. Generally speaking the smaller leaves are deeply cordate, and with increase in size there is a tendency toward a flat oblique base.

One fossil species, *Tilia populifolia* Lesq., represented in the Fort Union and Florissant floras, was found to have a counterpart in several leaves from adventitious shoots of *T. americana*. The tip of the figured fossil specimen is broken off, but from its description and the figures one is able to compare the fossil and living species.

Ulmus americana Linn.

Leaves of *Ulmus americana* which were abnormal were collected from shoots which had sprung up around old stumps after the trees had been cut down. The leaves varied in shape and size, but were similar in several major respects. They seem to have lost the characteristic obliqueness of *U. americana*, are coarsely dentate, and are of a thin texture.

Very few of the abnormal leaf types of *U. americana* were found to be represented in the various reported species of fossil *Ulmus*. The reason for this is believed to be that since a major characteristic of the normal leaves of this species is their obliqueness, some fossil species were not recognized as such because of lack of this character.

It is the general contention that plants whose leaves are injured in some way will put out new ones which are usually unlike the normal leaf and which represent a reversion to the ancestral type. If this is true in the case of the *Ulmus* leaves under discussion, there are probably many leaves of the genus present in the fossil record which have never been recognized as such because they do not exhibit the characteristics so well shown in the normal leaves.

The abnormal leaves show a marked similarity to those of *Zelkova ulmoides* Schneider of southwestern Asia and *Zelkova oregoniana* (Kn.) Brown, a Mascall formation, Miocene species. This may mean that *Zelkova* and *Ulmus* had a common ancestor.

Platanus occidentalis Linn.

Several trees of *Platanus occidentalis* have been planted in the nursery at Carleton College in Northfield, Minnesota. Owing to the rigorous climate, these trees die to the ground each winter and send up new shoots each spring. The leaves vary greatly in size and shape. The large trilobate leaves are found near the tips of the shoots, while the small leaves which have a tendency away from the lobing, are found near the base of the shoots. The leaves from these shoots were collected and compared with the various fossil species of *Platanus*. Five fossil species, which ranged in age from the Cretaceous through the Miocene, were found to be referable to living species.

Geologic ages of fossil species referred to *P. occidentalis:*

P. dissecta	Miocene
P. guillelmae	Eocene
P. haydenii	Eocene
P. latior grandidentata	Cretaceous
P. raynoldsii	Eocene

Platanus dissecta Lesq. (11)

Platanus dissecta exhibits the same variation shown in the modern leaves. According to BROWN's figures (1) the species includes leaves which vary in form from trilobate to unlobed, as do the living leaves. The leaves of the living species agree with *P. dissecta* in all details except that the angle of the secondaries is much greater in the living species. However, there is so much variation in this point, the smaller unlobed modern leaves exhibiting the angle characteristic of the fossil species, that it is believed the living leaves are referable to this fossil species. The living leaves have a cuneate base with the first primaries arising well above it. This characteristic is present in the fossil species but is not constant.

Platanus haydenii Newb. (15)

The atypical leaves of *Platanus occidentalis* under consideration vary in form from deeply trilobate to an almost complete absence of lobing. Those which are lobed generally have a dentate margin, and the primary and secondary veins arise at the midrib, forming a wide angle. The leaves which are not trilobate have a tendency toward a doubly dentate margin and the veins form a much more acute angle with the midrib. The fossil species represents a composite of the two types. While no one leaf can be referred to the fossil leaves figured, the group as a whole has the characteristics of the fossil species—the acute angle of the primary and secondary veins and the simple dentation. NEWBERRY (14) has pointed out that this fossil species might be one of the ancestors of *P. occidentalis*. "In general aspect the species now before us is more like the eastern than the western of our American sycamores, to the former of which it has considerable likeness and may very well have been its progenitor."

Platanus latior (Lesq.) Kn. (**12**)

The nervation and shape of this fossil species resemble closely some of the atypical leaves of *Platanus occidentalis*. There is one point at which there is a lack of similarity, the margin. None of the atypical leaves exhibited such blunt, simple, widely spaced teeth as characterize the fossil species. LESQUEREUX referred to the great similarity of the fossil species to *P. occidentalis*, and for that reason it is included in this report. "Indeed, comparing the leaves of *P. primaeva* [*P. primaeva* Lesq. = *P. latior* (Lesq.) Knowlton] of the Dakota group with those of *P. haydenii* of the Laramie, those of *P. guillelmae* of the old Pliocene of California, and those of the living *P. occidentalis*, one sees the original type so clearly and distinctly preserved that, overlooking the great difference in age, it would be difficult to separate these into different species" (**13**). The atypical leaves of *P. occidentalis* show marked similarity to *P. latior grandidentata* (Lesq.) Knowlton, and are probably referable to that species.

Platanus guillelmae Göppert (**8, 20**)

As already stated, LESQUEREUX (**13**) remarked on the similarity between the fossil species and *P. occidentalis*. The leaf specimens here under consideration may be referred to some figured leaves representing this fossil species (**8**), but they are not similar to the one fossil figured as representing the same species which WARD (**25**) states most nearly resembles the type fossil of the species figured by GÖPPERT.

Platanus raynoldsii Newb. (**17**)

NEWBERRY describes the general character of the nervation and leaf as a whole as similar to that of *Platanus occidentalis*. The great difference is in the margin, that of the fossil species being characterized by being doubly dentate. Some of the atypical leaves of *P. occidentalis* exhibit the same characteristic, so it is here compared with this fossil species.

Summary

If the normal leaves of trees and shrubs are destroyed, the leaves which subsequently develop are usually atypical. When these

atypical leaves are compared with fossil species belonging to the same genus, a great similarity is noted. The characteristics of the atypical leaves of the living species are found represented in the fossil species, and for that reason the atypical leaves are believed to be atavistic; that is, to represent a reversion to an ancient type present during the growth and development of that species in the past.

This study was made under the direction of Dr. A. C. Noé of the University of Chicago, to whom the writer is greatly indebted.

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MAJOR CHANGES IN GRASSLAND AS A RESULT OF CONTINUED DROUGHT[1]

J. E. WEAVER AND F. W. ALBERTSON

(WITH NINE FIGURES)

Introduction

A comprehensive research on 135 large tracts of prairie was completed in 1933 after five years of study. These tracts were distributed over an area of 60,000 square miles, including the eastern one-third of Nebraska, the western one-third of Iowa, and adjacent areas in the four neighboring states (**2**). This investigation furnished the background for an understanding of the profound changes which have occurred during the several years of the great drought, which first became pronounced in 1934. The response of prairie to the drought of that year has been discussed (**3**). Likewise, a detailed account of the destruction caused by the drought years of 1934 and 1935 and resultant changes and shifting of populations in both true and mixed prairies have been recorded (**1**).

Research has been continued from season to season throughout the long period of abnormally low rainfall. Continuous records of climatic and edaphic factors have been obtained, and changes in behavior and structure of vegetation intensively studied. These have been recorded in detail by means of scores of permanent quadrats scattered widely from eastern Nebraska to western Kansas. With successive years of drought further deterioration has occurred. Favorable precipitation during the spring and early summer of 1938 (and during 1937 in certain portions of the area) has brought about important early stages in recovery. Several years with normal or excess precipitation, however, must intervene before the general trends toward readjustment are clearly seen. The purpose of the present paper is to summarize some of the more important changes which have occurred since 1935.

[1] Contribution no. 110 from the Department of Botany, University of Nebraska.

Observations

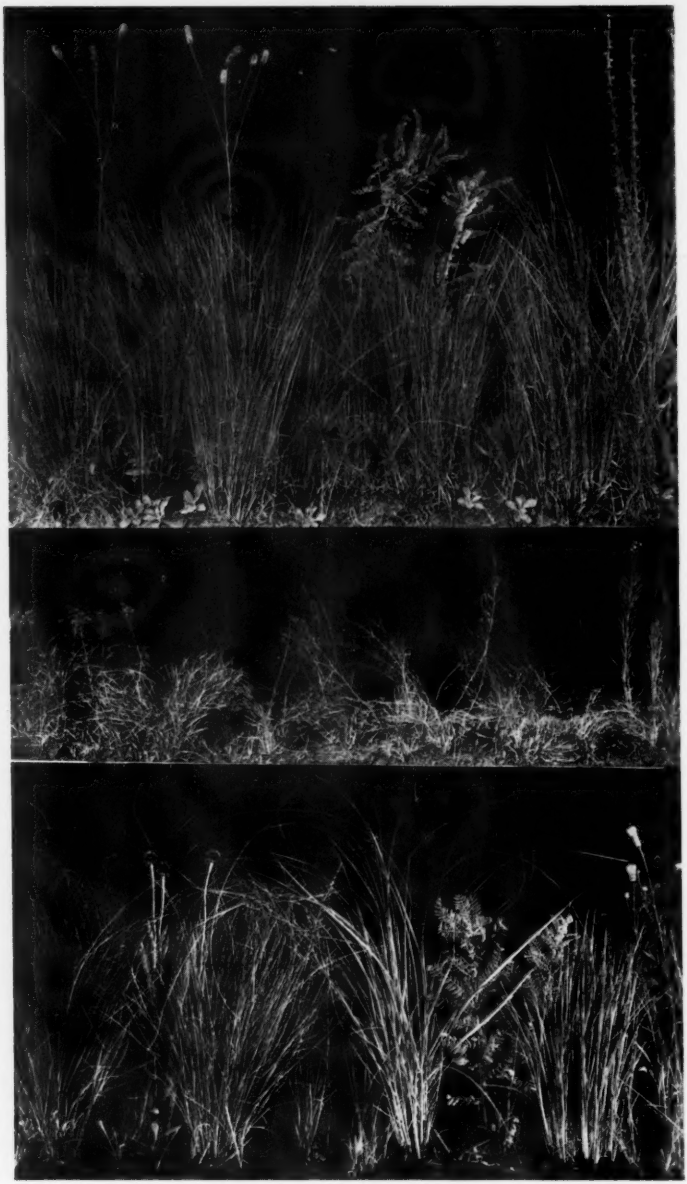
Decrease in perennial grasses

Among the most striking effects of the drought is the very great depletion of the formerly most important regional dominant, little bluestem (*Andropogon scoparius*). In numerous prairies not a trace remains; in others small amounts persisted, usually only in the most favorably moist habitats (figs. 1, 2, 3). This loss has been general in true prairie of the southern half of Nebraska. In Iowa this grass has generally persisted but sometimes with a shifting in importance from first to third place, being outranked by *Sporobolus heterolepis* and *Andropogon furcatus*. Since this species alone frequently occupied 50 to more than 70 per cent of the total basal cover on uplands, its death has left open great areas for invasion.

The loss of Indian grass (*Sorghastrum nutans*) has been similar to that of little bluestem. Its nearly complete disappearance is much less noticeable, however, since it formerly constituted only about 2 per cent of the basal cover of vegetation.

The death of Kentucky bluegrass (*Poa pratensis*) has been general in pastures, where it often had nearly complete control, but was somewhat less in prairie because of the shade afforded by mid grasses and forbs. While many prairie areas (especially westward) are practically free of this invader, which normally furnished 5 to 9 per cent of the basal cover on uplands and lowlands respectively, in others it occurs commonly in ravines and at the bases of north slopes. During the favorable season of 1938, these relict patches produced a fine crop of seed and also spread rapidly vegetatively.

That the tall grass, *Andropogon furcatus*, should persist in mixtures where the mid grass, *A. scoparius*, died seems anomalous, but the reason is readily explainable. As the soil moisture became depleted from above downward, little bluestem succumbed when its root system, which is usually about 4 feet deep, had exhausted the available moisture to this depth. The larger and more deeply rooted big bluestem, although much desiccated, persisted. But since the light rainfall of the following years did not add to the scant supply of subsoil moisture, the big bluestem also often succumbed. Its decrease in abundance in 1936–37 has been repeatedly recorded. Thus



FIGS. 1–3.—Upland little-bluestem prairie at Lincoln, Neb., early in August in the same square rod but during different years: Fig. 1, normal stand in 1932 of *Andropogon scoparius* 18 inches tall. Note lower story of *Antennaria campestris* and *Panicum scribnerianum*, and upper one of *Petalostemon candidus* (left), *Amorpha canescens*, and *Liatris scariosa*. Fig. 2, dried bluestem in 1934 only 5 to 6 inches tall and forbs about half normal height. *Solidago glaberrima* (left), *Aster multiflorus*, and *Liatris punctata*. Fig. 3, remaining bluestem (right) in 1938, 12 inches tall; rest has been replaced by *Sporobolus heterolepis* (bunches on left) and *Stipa spartea* (center). Forbs are *Echinacea pallida* (left), *Amorpha canescens*, and *Petalostemon candidus*. Note wide spacing of grasses, complete disappearance of lower layer, and bareness of soil.

over large areas in certain prairies now occupied by needle grass (*Stipa spartea*) or other xeric species, big bluestem has largely vanished from the upland just as little bluestem disappeared during the earlier years of the drought. But this vigorous species, never entirely routed from the uplands, where it formerly constituted as much as 15 per cent of the basal cover, made a remarkable growth during 1938. Having much moist, rich soil with little competition, it reached a height and stem diameter otherwise attained only on lowlands, flowered profusely, and spread rapidly by means of strong rhizomes. It is thus claiming much territory formerly occupied by little bluestem, with which it was so regularly intermixed.

General decrease of forbs

The recurrence of years with minimum rainfall has resulted in great decrease of most species of forbs. This pertains to those that root deeply as well as to those of moderate and shallower rooting habit. Some evidence has been obtained that certain species persisting on subsoil moisture at depths below 4 or 5 feet extended their roots even more deeply. But although moisture below the solum was continuous to great depths, repeated sampling showed that it existed only in small amounts. Throughout the drought years rainfall did not augment the supply. Consequently the roots of most individuals came in contact with only definitely limited amounts of deep soil moisture, and many were poorly fitted to profit from water occasionally furnished by rainfall to the surface soil. These conditions, often coupled with desert-like environment aboveground, readily account for the death of increasing numbers of forbs. *Astragalus crassicarpus*, *Psoralea floribunda*, *Helianthus rigidus*, and *Kuhnia glutinosa* furnish examples of the general situation. Their disappearance was much more gradual and later than that of the shallowly rooted *Fragaria virginiana*, *Antennaria campestris*, and others, which were nearly swept away during the first year. To appreciate fully the paucity of forbs one needs only to examine the prairies eastward across the Missouri River, where they maintained almost their usual abundance. Elsewhere, with exceptions to be noted, losses have been approximated at one-half to two-thirds.

Behavior of certain annual grasses

Following the death of the perennial grasses during the first years of the drought, certain annual species became a veritable scourge. Chief among these were six-weeks fescue (*Festuca octoflora*), chess (*Bromus secalinus* or closely related species), and to a lesser extent little barley (*Hordeum pusillum*). These were nature's shock troops which held the bared soil from eroding until reclaimed by a more stable population. While *Festuca* is still plentiful in most drought-swept true prairies and *Bromus* and *Hordeum* in the western edge, yet none of these are now so continuous in the cover. As will be shown, the major bared or semi-bared areas have been so largely reclaimed by long-lived grasses or by certain native forbs that these annual grasses chiefly fill the smaller interspaces and are not found in large belts or patches as formerly.

Decrease of ruderals

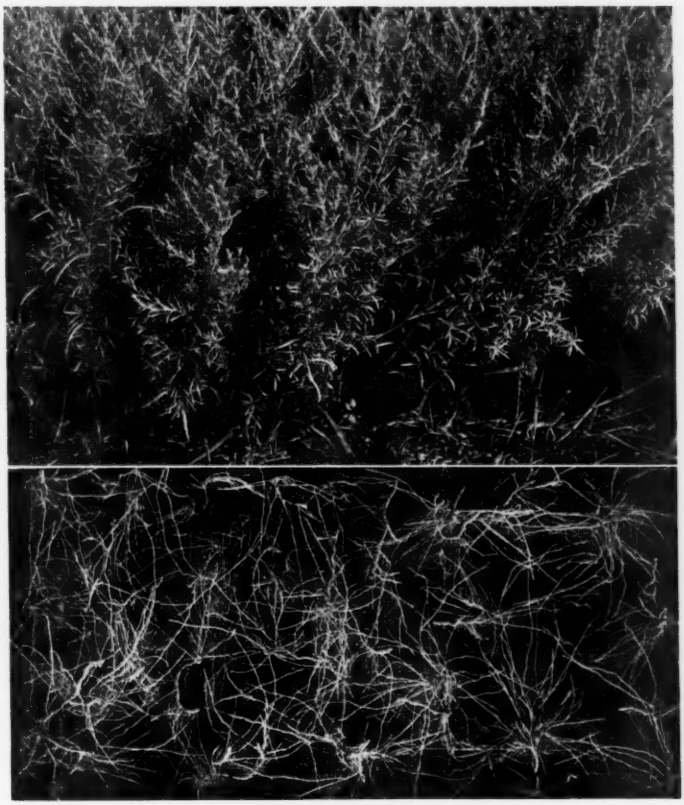
The scourge of pepper grass (*Lepidium virginicum* and *L. apetalum*), so serious in 1936 and 1937 from the Missouri River to the Rocky Mountains, was not in evidence in true prairie during the past year of abundant rainfall. The horseweed (*Leptilon canadense*) likewise persisted in great abundance for only a year or two and has now practically disappeared from the native grassland. More local serious infections of goat's beard (*Tragopogon pratensis*) have now all but vanished. Indeed the absence of ruderals in all the true prairies, except a few where the vegetation was buried under drifting dust, was an outstanding characteristic, especially during 1938.

Response of certain native forbs

The chief native forbs that profited by the death of their competitors were *Aster multiflorus*, *Erigeron ramosus*, *Solidago glaberrima*, and (during the first years of drought only) two annuals, *Silene antirrhina* and *Specularia perfoliata*. The last two increased from a ranking of rare or infrequent to one of extreme abundance, populating the drought-bared areas everywhere.

Aster multiflorus, propagating both by seeds and very efficient rhizomes, became so extremely abundant as to ruin many of the prairies or large portions of them for the production of hay (figs. 4,

5). In fact, during 1935–36 it appeared that this forb would completely dominate the prairie. As a result considerable native sod was



FIGS. 4, 5.—Fig. 4, edge of dense patch of *Aster multiflorus* about 2 feet tall. Fig. 5, roots and rhizomes of *Aster* obtained by washing away the soil of one-half square meter area to depth of 10 cm. Photographed Lincoln, Neb., July 25, 1938.

broken because of the seriousness of this pest. Plants with fifty or more woody stems with a spread of tops of 2.5 feet arose from a soil area of one-fourth square foot. Because of the low light intensity (often only 1 to 5 per cent of full sunshine at noonday), seedlings

grew with difficulty, and relict grasses, if present, were greatly attenuated. While *Aster* is still important in occupying many local areas in nearly pure stands, it is plainly waning. In semi-sodded areas a more open type prevails, that is, one to four stems in a place separated from others by rhizomes 2 to 6 inches long. Thus it approaches more nearly its pre-drought condition, where it ranked in general as the third most important upland forb. There was a great decrease in both size and abundance of this forb in 1937, and markedly so in 1938.

The enormous increase of *Erigeron ramosus* in most of the true-prairie areas is one of the outstanding features of the drought period. Always of high rank and great abundance, this typically short-lived biennial thrived in bare areas everywhere. Not only were the plants abundant but also often of greatly increased stature. Profiting by the numerous interspaces between the remaining bunches and mats of grasses, in eastern Nebraska especially, they offered a profusion of blossoms which at a distance appeared as newly fallen snow. *Erigeron* as a forb released from competition and persisting through the drought is an impressive phenomenon. In many of the western true prairies this species scarcely occurs.

Solidago glaberrima has increased greatly in many prairies, and its abundance is revealed especially in July with the yellowing of the inflorescence. One can easily anticipate the degree to which the grasses have been destroyed, that is, the bareness of the soil beneath, by observing the density of the stand of goldenrod.

It is of interest that, in the prairies of Iowa, where the losses by death were much smaller, in general, replacement was not by invading ruderals but by shiftings in the prairie populations. Increase of certain forbs, notably the rhizomatous *Coreopsis palmata* and to a less extent *Aster multiflorus*, was marked. The xeric grass, *Sporobolus heterolepis*, also made gains.

Response of certain forbs with large storage organs

A remarkable increase of certain species, formerly of little importance, in the bared areas has occurred during the last two years. Their wide distribution and dense aggregation were most marked during the moist spring and early summer of 1938. All are char-

acterized by some type of underground storage. In approximate order of abundance, the most important were *Oxalis violacea*, *Senecio plattensis*, *Tradescantia bracteata*, *T. occidentalis*, and *Delphinium virescens*. *Allium mutabile*, *Anemone caroliniana*, and *Erythronium mesochoreum* were also unusually abundant during the drought, although more locally distributed than the preceding. *Oxalis* formed

FIG. 6.—*Oxalis violacea* and *Agropyron smithii* invading drought-bared area. Photographed Crete, Neb., May 22, 1938.

dense patches, usually a few square feet or square yards in area, but such communities were widely distributed in most of the prairies studied (fig. 6). The somewhat fleshy rooted *Senecio*, formerly occurring as scattered individuals or small groups, increased manyfold and was a most conspicuous feature of the vernal aspect. Great groups of the fleshy rooted spiderwort occupied bared areas or were interspersed in the open places between the widely spaced bunch grasses. The thick-rooted larkspur had increased greatly in abundance and in places these usually solitary plants were rather closely

grouped. These species, like the wild onion, wind flower, and dog's tooth violet, had all been favored by a marked decrease in the competition of other prairie components.

Increase of wheat grass and certain other xeric grasses

The great losses sustained by *Andropogon scoparius*, *Poa pratensis*, and other species made possible the enormous increase of more drought-resistant, competing species as well as great invasions by *Agropyron smithii*. The latter phenomenon was by far the most striking feature of the drastic modifications of the prairie.

Extensive earlier surveys showed clearly that western wheat grass, although a dominant of other portions of the true prairie, notably the hardlands of the Dakotas, constituted scarcely any part of this association in the central prairie region. It occurred along roadways, occupied the compacted soil by the sides of trails through the prairie, and was found in abundance locally where a shallow claypan made conditions unfavorable for the growth of the usual dominants. Occasionally a few stems were found about disturbances such as gopher mounds. In native pastures, centers of infestation often occurred on knolls or about gateways where cattle or horses congregated and trampled the soil while stamping to free themselves of flies.

Immediately after 1934, wheat grass became increasingly abundant in both pastures and prairies. During the drought years it has continued spreading over all types of terrain, until today it is often the most important species in many prairies to within 40 miles of the Missouri River. Large circular areas where other species are practically absent indicate the excellent and rapid method of migrating by long, much branched rhizomes. Long narrow belts of wheat grass around the brows of hills indicate where early drought was most severe. Often whole hillsides are covered with pure stands of this grass, and many lowlands are a continuous area of undulating wheat-grass stems. Westward, grasslands hundreds of acres in extent have been three-fourths claimed by this species, and near Lincoln, Nebraska, similar extensive invasions of former bluestem prairies are easily found. Alternes or mixtures occur, but more often the stand is nearly pure. With often a thousand stems per square

meter and a height of 3 to 3.5 feet, this expanse of grassland is an impressive sight (fig. 7). Studies along the invading fronts and the thinning of the stands in some areas with an increase in former dominants give some evidence of a decrease of this rapid spread. But should drought years continue, wheat grass may become even more extensive.

FIG. 7.—Complete disappearance of *Andropogon* and invasion by *Agropyron smithii* (dark) near Jamaica, Neb. Belt of bluestems 2 to 5 rods wide borders postclimax forest along the stream for 80 rods, where it was protected from the hot, dry winds.

Stipa spartea, although at first retarded, has greatly profited by the drought. Its spread has increased enormously and many new centers have been established. This change has been most pronounced in prairies of eastern Nebraska, where invasion by wheat grass has not occurred or has been less extensive. At Nebraska City, for example, a great ridge extends northeastward across a 160-acre prairie. In 1928, only the northern crest of the ridge was dominated by *Stipa*, although it occurred as a species of much less importance than the bluestems along the entire ridge and extended also over the upper slopes. The early death of little bluestem was later followed by great losses of the more deeply rooted big bluestem.

Death of the andropogons, forbs, etc., proceeded gradually down the slopes and the area is now claimed by needle grass. A community of *Stipa* of great extent, with the single dominant furnishing 90 to 98 per cent of the cover of grasses, extends continuously along the crest and spreads far down the slopes. Only near the foot of the hills does one find the bluestems well intact and seriously resisting the down-

FIG. 8.—Open stand of *Stipa spartea* exposing much bare ground. Large portions of these bare areas recently reclaimed by seedlings of this species. Photographed June, 1937.

ward spread of needle grass (fig. 8). Had one not known the composition of the previous cover, the invasion still is clearly evident. Everywhere the cover is open and the soil bare. Only relatively few of the bunches of *Stipa* are large; most are young, small, and widely spaced. Seedlings of needle grass with only a few stems are everywhere, but they are firmly established and only the single stemmed new seedlings may succumb. As many as fifty to sixty small bunches of *Stipa*, one or two large ones, and numerous seedlings are common in a square meter. Similar although less extensive spreading of *Stipa* has been recorded elsewhere.

Prairie dropseed (*Sporobolus heterolepis*) is another xeric species that has spread considerably, especially in eastern Nebraska and western Iowa. In a few prairies it has become the ranking dominant; in many others its importance has greatly increased. *S. asper* has likewise become far more common and sometimes very abundant since the advent of the drought.

There has been a great spreading of the preclimax areas of short grasses. *Buchloe dactyloides*, with the death of its mid-grass competitors, has spread widely along the western fringe of true prairie and *Bouteloua gracilis* wherever it formerly existed, even if in small amounts. The latter was always of greater abundance in uplands and is somewhat more xeric. Increases in area of ten to fifteenfold have been recorded and new outposts have been established in many places. *Carex pennsylvanica*, which is similar in stature to short grasses, has likewise shown marked increases in many prairies and has greatly augmented its importance in pastures.

A grass that formerly occurred sparingly in almost all types of prairie but never attained complete local dominance is side-oats grama (*Bouteloua curtipendula*). During the years of drought it has spread widely until now it is one of the more important of prairie species. It has often persisted where all other grasses succumbed. Its remarkable increase has been due in part to its prolific seeding habit and in part to propagation by rhizomes. In many places it has largely replaced the losses occasioned by the death of little bluestem. In certain southwestern true prairies it has filled in locally bared areas with a pure stand. The bunches are often remarkably large, 6 or more inches in diameter, and the rhizomes 2 to 5 inches long. Elsewhere it often forms half or even more of the prairie cover. Thus the prairie patterns have changed in a most remarkable manner.

Reduction of basal cover, loss of lower layer and bareness of soil

The great reduction of the basal cover of the prairies generally, except in those relatively small areas where even continued drought left the former prairie carpet intact, is of wide occurrence. It has been a process of eliminating species less fitted to withstand drought, younger plants not yet deeply rooted, and (probably) less vigorous

individuals. From well distributed quadrats, plot estimates, and extensive observations, it has been determined that in general there has been a decrease of basal cover in most drought-stricken areas of 50 to 66 per cent. Small bare areas of one-half to one square foot occur regularly, while larger ones of 1 to 15 square meters with a basal

FIG. 9.—Representative bare area caused by continued years of drought. A few of the grasses (*Andropogon*) remained alive; others (*Elymus canadensis*, *Panicum scribnerianum*) are invaders. The forb is *Tradescantia bracteata*. Photographed May 22, 1938.

cover of only 1 per cent are frequent (fig. 9). Where early summer rains were abundant, this wide spacing permitted an excellent growth of foliage. The bunch or sod was filled with stems; the foliage had spread widely. Each species alone produced enough seed to reclaim the entire area. Such a rank growth affords a foliage cover of 70 to 100 per cent. From the same base it may be only 30 to 50 per cent during a dry season. Hence one may be misled and believe that recovery has progressed far beyond the real state. Actually there is room for invasion almost everywhere.

One who knows the prairies of old is constantly impressed with the bareness of the soil. The rank growth of a well developed three-layered vegetation is no more. The ground layer of mosses, lichens, *Antennaria campestris*, low species of *Panicum*, *Viola*, *Astragalus crassicarpus*, and tufts of *Poa* has practically disappeared. Nor is the soil clothed with the former mulch of fallen leaves, flower parts, stems, etc. Much less of this material has been formed; the lack of moisture and abundance of direct sunshine and wind have caused it to wither and dry or often to blow away, leaving the black soil quite bare. Exceptions are the areas thickly sown by the wind to *Festuca octoflora*, etc., where the stems, lodged, dead, and dried by midsummer, protect the bare soil from sun, wind, and flood.

Both crowns and roots of the dead grasses decayed slowly because of the arid conditions. But after a few years an unusual abundance of dead organic material filled the prairie soil. This promoted the growth of numerous fungi, among which the red stinkhorn (*Simblum sphaerocephalum*) was most abundant and important. During the moist spring and early summer of 1938 this species was found widely and usually thickly distributed in nearly all the prairies. Ten or more individuals per square meter were not uncommon on low ground, and often the air was foul with the nauseating odor produced by this saprophyte.

Modification of grassland patterns

Still another drought feature of the landscape is the sharpness of the alternes marked off as a result of the great destruction, readjustments, and invasions. Here a deep soil area is so located that it escaped the full force of the oven-hot wind; vegetation remained intact. A pace beyond death has struck and *Stipa* or *Sporobolus* has spread to claim the area, or *Koeleria* has repopulated it, or perhaps *Agropyron* is in complete possession. The front and flanks of the mass movement of the latter can clearly be traced usually to within 1 meter, for great distances. Or one may in a step or two pass from pure stands of *Andropogon furcatus* into those of *Bouteloua curtipendula* or even *B. gracilis*. Large circular or irregular areas temporarily dominated by *Aster* or *Erigeron* give way abruptly to a fine growth of *Andropogon furcatus*, or patches of pure *Festuca* alternate with

bare places, as if nature could not in such extremity reseed the whole bare surface. Such sharp transitions are remarkably different from the former intimate intermixtures of climax dominants and the broad ecotones separating the several types. Some of these remain, of course, but in general the change is outstanding.

Signs of recovery

The past year has witnessed an enormous crop of seedlings scattered widely throughout the prairies. Prominent among these have been the grasses. *Bouteloua curtipendula*, *B. gracilis*, *Panicum scribnerianum*, *Andropogon furcatus*, *Koeleria cristata*, and *Stipa spartea* were especially well represented. Seedlings of *Erigeron ramosus*, *Aster multiflorus*, and *Senecio plattensis* ranked highest among the forbs, although a good population of various species of *Psoralea*, *Liatris*, *Kuhnia*, and others was found. Moreover, the few survivors among species of the lower layer are reviving and spreading gradually. Mat plants, bluegrass, and other low-growing forbs and grasses are beginning to reconstruct this layer next to the soil. A few prairies in certain sections favored by two consecutive years of good rainfall are started toward recovery. Although late summer drought destroyed much of the new seedling population, some survived, and with the enormous seed crop of the current year they will repopulate the bared areas if and when good rains prevail.

Summary

1. As a result of the great drought of 1934–37, the most important upland dominant, *Andropogon scoparius*, has suffered great destruction in the southern half of Nebraska.

2. The more deeply rooted *Andropogon furcatus* persisted during the early years of drought but has since suffered heavy losses.

3. *Poa pratensis* was nearly all killed in bluegrass pastures and only relatively small amounts remain in prairies.

4. Most species of forbs, including those very deeply rooted, have gradually succumbed to the continued drought, until they are only one-half to one-third of their former abundance.

5. *Festuca octoflora*, *Bromus secalinus*, and other annual grasses, which became extremely abundant after 1934, have now greatly

decreased in quantity, filling the smaller interspaces rather than occupying large areas.

6. The scourge of the ruderal, *Lepidium virginicum*, so serious in 1936–37, has distinctly disappeared. Other annual weeds were extremely abundant only during one or two seasons.

7. The perennial, rhizomatous *Aster multiflorus* spread so widely into drought-bared areas as to ruin many prairies for production of hay. *Erigeron ramosus* was almost equally widespread.

8. A remarkable increase in abundance of *Oxalis violacea*, *Allium mutabile*, *Tradescantia bracteata*, and other species with thick roots or other storage organs has recently occurred.

9. *Agropyron smithii*, occurring sparsely at the beginning of the drought, has spread so widely as to cover one-half to three-fourths of the area of many former bluestem prairies.

10. Marked increases in territory dominated by *Stipa spartea* and *Sporobolus heterolepis* have appeared. *Bouteloua gracilis* and *Buchloe dactyloides* have greatly increased. *Bouteloua curtipendula*, a dominant, formerly of relatively low rank, has now become one of the most important prairie grasses.

11. Drought has reduced the basal cover in true prairie 50 to 66 per cent. The lower layer of grasses and forbs has been almost destroyed. Grassland types have been much modified.

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INCREASING THE FERTILITY OF NEUROSPORA BY SELECTIVE INBREEDING[1]

CARL C. LINDEGREN, VIRGINIA BEANFIELD, AND ROBERTA BARBER

(WITH ONE FIGURE)

Haploid ascospores of *Neurospora crassa* germinate to produce either of two mutually exclusive kinds of mycelia, (+) or (−). The sexuality of mycelia can be determined only by mating with a standard tester strain and noting whether or not fertile perithecia (containing ascospores) result. When grown alone neither of these types of mycelia is capable of producing perithecia which contain ascospores, but either a (+) or a (−) mycelium grown alone produces many small sclerotia or bulbils. DODGE (1) has shown that these structures usually cease to grow after attaining a size of about 50 μ, unless they are spermatized, when they develop into perithecia full of ripe ascospores. However, in some unisexual mycelia a considerable number of these tiny bulbils continue their growth until they attain the size of a mature perithecium, thus proving that the growth of the perithecial wall does not depend entirely on bisexual heterokaryosis. None of these unfertilized structures ever contains either ascospores or even rudimentary asci. Asci (even those containing aborted ascospores, 8) are produced only when (+) and (−) strains are mated.

In the standard test for sex, a (−) tester strain is grown together with the tested strain. If perithecia containing ascospores result, the tested strain is labeled (+). However, if the (+) mycelium being tested contains sterility factors which prevent production of ascospores, the mating would fail and the (+) mycelium would be mistaken for a (−) mycelium. It is best therefore to cross an unknown strain with both (+) and (−) tester strains and to withhold judgment unless one cross produces ascospores and the other fails. Often a mating of (+) by (−) produces only sterile perithecia, but in much

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greater abundance than that produced by either unisexual strain grown alone. Occasionally when two mycelia of the same sex are grown together there appear to be more sterile perithecia produced than when either is grown alone.

The fertile inbred strains of *Neurospora crassa*[2] which have been selected in this laboratory (**3, 4, 6, 7**) show considerable variation in the perithecia and asci which they produce. When (+) and (−) *fluffy* are mated, numerous perithecia containing an abundance of asci filled with ripe ascospores are produced. Millions of ascospores are ejected from the perithecia on to the walls of the test tube, so that it looks as if it were covered with soot. When perithecia about a month old are crushed, only a few unripe asci can be found in each cluster. However, about one-fifth of the perithecia are empty. The fertility of the *fluffy* strain is so great that when it is mated to a less fertile strain of opposite sex, more ascospores are produced than that strain normally produces if inbred. By contrast to *fluffy*, the *pale* stock produces fewer ascospores. *Pale* by *pale* crosses produce great numbers of perithecia, but most of them are sterile and can be recognized macroscopically by their light brown instead of jet black color. Fertile perithecia have dense black masses of ascospores piled at the ostiole. Sterile perithecia viewed from above show the opening of the ostiole as a white area surrounded by a darker ring. The fertile perithecia of a *pale* by *pale* cross do not contain an abundance of asci filled with ripe ascospores, but when the perithecia are crushed each cluster contains many asci in which unripe or aborted ascospores are found. Microscopic inspection of the walls of the tube is necessary to reveal ejected spores. While both the *fluffy* and *pale* stocks produce many hundreds of perithecia in each test tube, crosses of *even* by *even* often produce only a few dozen perithecia in each test tube. However, these perithecia are usually filled with asci containing ripe viable ascospores.

Maximum fertility apparently depends on one mechanism which operates to develop perithecia from bulbils, and a second mechanism

[2] In studying cultural differences it is necessary sometimes to keep the cultures out in the laboratory for many weeks, often resulting in infestation with mites. This can be prevented merely by pulling the cotton plug two-thirds out of the tube, dipping it in glycerin, squeezing out the excess, and reinserting it.

which results in the production of ascogenous hyphae. Enlargement of the bulbils occurs when mycelia of opposite sex are grown together, indicating that heterokaryosis of (+) and (−) nuclei stimulates this increase in size. But the fact that growing two mycelia of the same sex together often causes the bulbils to attain the size of mature perithecia indicates that some of the genetical factors involved are independent of (+), (−) heterokaryosis. In the *fluffy* stock the enlarged bulbils are abundant and the ascogenous hyphae are particularly fertile. In a *pale* by *pale* cross the enlarged bulbils are even more abundant, but the ascogenous hyphae produce many aborted ascospores. In *even* by *even* crosses only a few enlarged bulbils are produced, but the ascogenous hyphae are exceptionally fertile.

The standard tester used in this laboratory is highly fertile. It was originally a homokaryotic *tan* strain (no. 1026-5) which reverted to *normal* (**3**, table 1). After it had been asexually propagated for several years it became heterokaryotic for at least three genetically different strains, *normal*, *peach*, and *clump*, owing to successive mutations (**6**). Although several mutations occurred during the long period of asexual propagation, the sex genes were unaltered. When this unisexual heterokaryotic mycelium is grown alone on cornmeal agar, it produces an abundance of sterile perithecia, some rather large. The *normal* heterokaryotic unisexual tester strain (1026-5) just described, like the *fluffy* strain, carries factors which compensate for low fertility of other strains to which it may be mated. A cross of *pale* by the tester contains numerous perithecia filled with ripe ascospores; a cross of *even* by the tester likewise contains numerous perithecia filled with ripe ascospores.

This tester strain can be used to diagnose the sexes of ascospores whose mycelia are almost completely sterile *inter se*. In earlier papers the mechanism of crossing over in the sex chromosome has been analyzed by the dissection of the eight spores from each of several hundred hybrid asci, and mycelia were produced by growing these ascospores in tubes by themselves. The progeny of these crosses carried so many sterility factors that numerous matings were necessary between many different pairs of known (+) and (−) mycelia to obtain a cross which produced ascospores for the next generation.

In each successive generation only the relatively more fertile

matings were analyzed. This inbreeding resulted in an increase of fertility in each succeeding generation. The relative increase in fertility of four generations is reported here. All these mycelia were

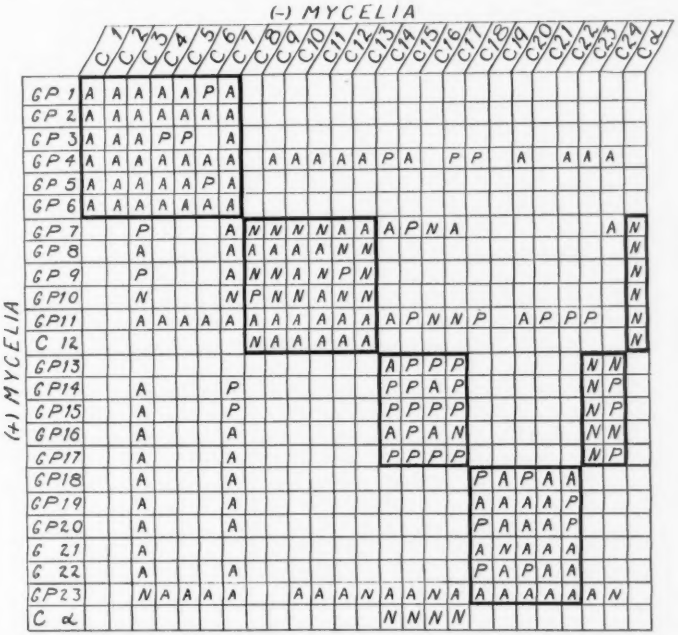

FIG. 1.—Fertility of matings between 23 (+) and 24 (−) mycelia in fourth inbred generation: *A*, fertile perithecia containing ascospores; *P*, sterile perithecia; *N*, no perithecia. Matings blocked in the rectangles were made first, then the mycelia which these first tests showed to be highly fertile were mated with all the others. In this second test 69.4 per cent produced fertile perithecia. Cα was sterile in all matings tested, so its sex is unknown. Second four *N* matings of Cα not used in calculating percentage fertility.

tested against the highly fertile tester strain to determine their sexes. Then the strains were mated. Since the sexes were known, only (+) and (−) matings were made. In each ascus there are four (+) and four (−) spores. The mycelia of each of the two (−) genotypes were mated with the mycelia of each of the two (+) genotypes in forty-one asci obtained from three successively inbred genera-

tions. In the fourth generation the spores were selected at random and neither the ascus nor the positions in the ascus of the different ascospores were known. Although it had already been established that in each mating the two given strains were respectively (+) and (−), many of the matings failed to produce fertile perithecia.

The first generation was descended from a cross of (+) *gap, pale* (+GP) by (−) *crisp* (−C). Six per cent of the matings produced ascospores. In the second generation (+GP × −C) 30 per cent of

TABLE 1

FERTILITY OF FOUR SUCCESSIVE INBRED GENERATIONS

GENERATION	NUMBER OF MATINGS PRODUCING					
	FERTILE PERITHECIA (A)		STERILE PERITHECIA (P)		NO PERITHECIA (N)	
	No.	%	No.	%	No.	%
First	5	5.6	10	11.2	74	83.2
Second	15	30.0	8	16.0	27	54.0
Third	47	57.2	11	13.4	24	29.5
Fourth (random)	82	59.0	31	22.3	26	18.7
Fourth (selected)	50	69.4	13	18.1	9	12.5

the matings produced ascospores. In the third generation (+GC × −P) and (+GP × −C) 57 per cent of the matings produced ascospores. In the fourth generation, 59 per cent of the random matings produced ascospores. When the mycelia which this random test showed to be highly fertile were mated with other similarly fertile mycelia of opposite sex, 69 per cent of the matings produced ascospores (fig. 1). The results of these matings were classified as to whether they produced fertile perithecia, only sterile perithecia, or no perithecia (table 1).

The asci used were those reported by LINDEGREN and LINDEGREN (9). One of the commonest types found in dissection is the following: −C, −CP, +G, +GP. In such an ascus the following matings would be made: (1) −C × +G; (2) −C × +GP; (3) −CP

$\times$ +G; (4) −CP $\times$ +GP. The various mycelia originating from the spores of one ascus were mated as follows:

SPORES	GENOTYPE	MATINGS
1.....	−C	1×5
2.....	−C	2×8
3.....	−CP	3×5
4.....	−CP	4×7
5.....	+G	
6.....	+G	
7.....	+GP	
8.....	+GP	

There are five possibilities: (1) none of the matings may produce ascospores; (2) one of the four may produce ascospores; (3) two of the four may produce ascospores; (4) three of the four may produce ascospores; and (5) all of the four may produce ascospores (table 2).

TABLE 2

FERTILITY OF INTRA-ASCUS MATINGS IN THREE SUCCESSIVE GENERATIONS

GENERATION	NUMBER OF MATINGS FROM EACH SET OF FOUR INTRA-ASCUS MATINGS WHICH PRODUCED ASCOSPORES				
	NONE	ONE	TWO	THREE	FOUR
First.....	16	2	1	0	0
Second.....	3	5	3	0	1
Third.....	0	3	2	3	2

The smaller number of crosses analyzed in table 2 as compared with table 1 results from the fact that only in a limited number of asci were all four possible crosses made. Naturally the data in table 2 can be used only if all four crosses have been tried.

SHEAR and DODGE (10) reported that the matings they made between the different species of *Neurospora* produced ascospores, provided the matings were between mycelia of opposite sex. LINDEGREN (1929 unpublished) mated the separate mycelia from one unusual seven-spored ascus of *Neurospora tetrasperma* with six separate

mycelia from an eight-spored ascus of *N. sitophila*, making a total of forty-two matings. Only seven matings produced ascospores; twenty should have done so. Fertility was only 35 per cent. In a second set of matings the same seven mycelia of *N. tetrasperma* were mated to six different mycelia from an eight-spored ascus of *N. crassa*. Only three of the matings produced ascospores; 13.6 per cent fertility. Finally the six *N. crassa* and the six *N. sitophila* mycelia were mated in all combinations. Seven of the matings produced perithecia; eighteen should have; therefore 39 per cent of the matings were fertile.

In all these tests only a single tube was used. That is, if ascospores were not produced in the first mating made, the test is reported N or P. But a single mating with the highly fertile tester is always capable of producing ascospores when opposite sexes are used, so this arbitrary classification is valid. If many subsequent trials are made, however, either a P or even a N cross may produce ascospores in some tubes. Therefore the designation cannot be considered as completely sharp and clear cut. Possibly SHEAR and DODGE studied many different successive matings of the same pair of mycelia in their interspecific crosses or used strains of exceptional fertility.

Summary

1. Mutant strains of *Neurospora crassa* are often extremely infertile.

2. By selecting fertile strains and inbreeding, it is possible to increase fertility.

3. The development of the perithecium and that of the ascogenous hyphae seem to be controlled by independent mechanisms.

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PHLOEM DEVELOPMENT AND FLOWERING[1]

B. ESTHER STRUCKMEYER AND R. H. ROBERTS

(WITH EIGHTEEN FIGURES)

Introduction

WILTON and ROBERTS (2, 3) reported that the anatomy of the flowering plant is different from that of the non-flowering plant. They found a similarity in the anatomical structure of all flowering plants examined although a wide range of environmental treatments was needed to secure flowering. Observations with respect to the details of the anatomy of the phloem of flowering and non-flowering plants are presented in this paper.

Material and methods

Most of the plants used in this investigation were grown in the greenhouses and the gardens of the horticultural department of the University of Wisconsin. The plants in the greenhouse were grown under several light and temperature conditions (1). The responses to temperature and light were noted and samples were taken for an anatomical study of the stems. The non-flowering and flowering plants were planted at the same time and so were of the same age when sampled.

Samples of flowering stems were taken at the second internode below the inflorescence, and non-flowering stems were sampled at the third well developed internode below the tip of the shoot. To avoid the transition stages occurring between flowering and non-flowering stems, considerable care was taken to insure that samples of strictly vegetative and flowering plants were secured.

As a further check on the relation of the anatomical condition to flowering, several species of plants were pruned to induce new shoot growth from axillary buds. Some of these new shoots produced flowers, while others remained vegetative. Samples were taken of the internode below the node from which the new branches arose.

[1] Published with the approval of the Director of the Agricultural Experiment Station.

In order to obtain samples of non-blossoming stems, it was necessary to defoliate or place in the shade those plants which have a persistent tendency to flower. A number of plants such as tobacco, mustard, *Cleome*, and *Tropaeolum* which had been pruned were placed in the shade to provide an environment in which the regenerated shoots would not blossom.

The samples of the stems were fixed in formalin-acetic-alcohol, and normal butyl-alcohol was employed as the dehydrating agent. Cross sections of the paraffin imbedded samples were cut 12–18 μ in thickness. Safranin and light green proved satisfactory for staining these sections. Longitudinal sections were prepared to determine the presence of callose.[2] These sections were stained with China blue and iodine-potassium-iodide.

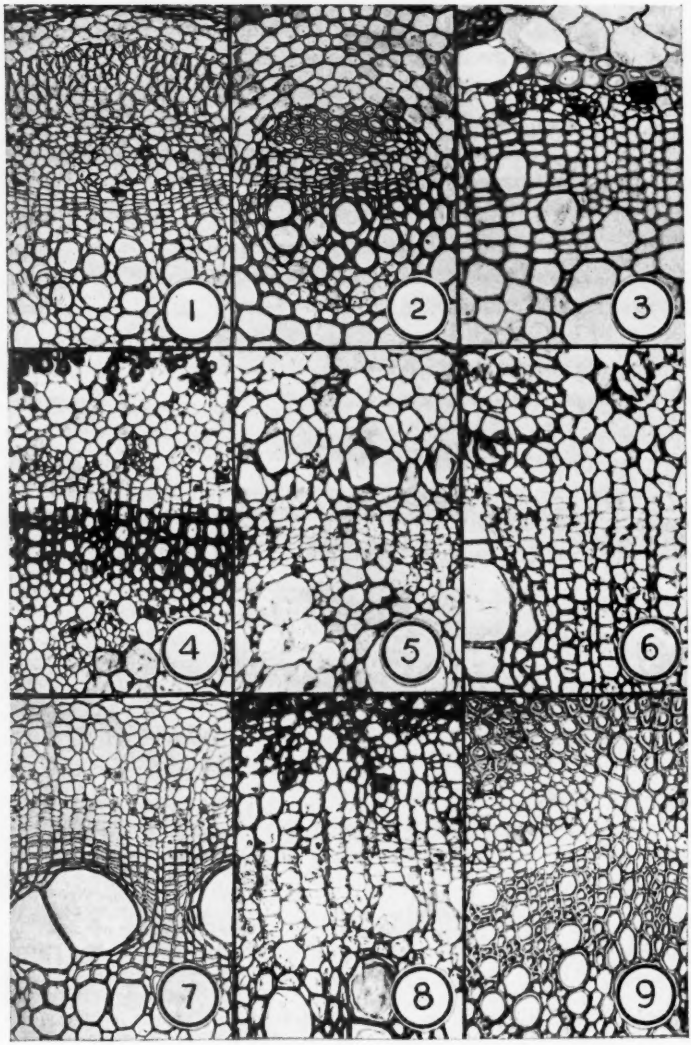
Observations and discussion

From the study of eighty-five species and varieties of plants it was found that the structural details of the phloem of the flowering and non-flowering plants are different. The phloem of the flowering stem differs from that of the vegetative stem in that there is a less amount of this tissue, the sieve tubes and companion cells are smaller, the walls of the cells are thicker, some of the cells in some species are crushed, parenchyma is formed adjacent to the cambial region, and more callose is present in the sieve tubes.

These details of the phloem are present in an unequal degree in flowering stems of different species. In each case, however, three or more of these characters are present which distinguish the phloem of the flowering from that of non-flowering stems.

A significant feature of the phloem of the flowering stems is the limited formation of sieve tubes and companion cells following the reduced cambial activity which precedes blossoming. *Ageratum*, *Zinnia*, *Dianthus*, *Hedalgo wercklei* (figs. 1, 2), and hemp illustrate

[2] The misuse of the term callus for callose has frequently occurred in literature. According to Webster, callus is the soft parenchymatous tissue which forms over any wounded or cut surface of a stem. When the wound occurs on stems exposed to the air, the callus soon forms a cork cambium. Callose, on the other hand, is a deposition on the cell wall, especially in sieve tubes, differing from cellulose in that it is insoluble in Schweitzer's reagent and soluble in soda solution. In addition it is easily colored by aniline blue and rosalic acid.



FIGS. 1–9.—Portions of transverse sections of stems: Figs. 1, 2, *Hedalgo wercklei* (1, non-flowering; 2, flowering). Figs. 3, 4, *Fagopyrum esculentum* and *Vinca major* (both flowering plants). Figs. 5, 6, *Ricinus communis* (5, non-flowering; 6, flowering). Fig. 7, *Ipomoea batatas* (non-flowering). Figs. 8, 9, *Tagetes erecta* (8, non-flowering; 9, flowering).

this condition. Attention was given to those plants which are difficult to keep from flowering and also to those which flower only rarely. *Fagopyrum esculentum* (fig. 3) and *Begonia semperflorens* flower more or less continuously under greenhouse conditions. It was observed that the phloem cells in these plants are fewer in number than in those plants which rarely flower. *Vinca major* (fig. 4), for example, flowers only very occasionally, and the cells of the phloem even in the flowering stems of these plants are relatively numerous. Also a plant such as the sweet potato (fig. 7), which could not be induced to flower under our cultural treatments, showed a large amount of phloem.

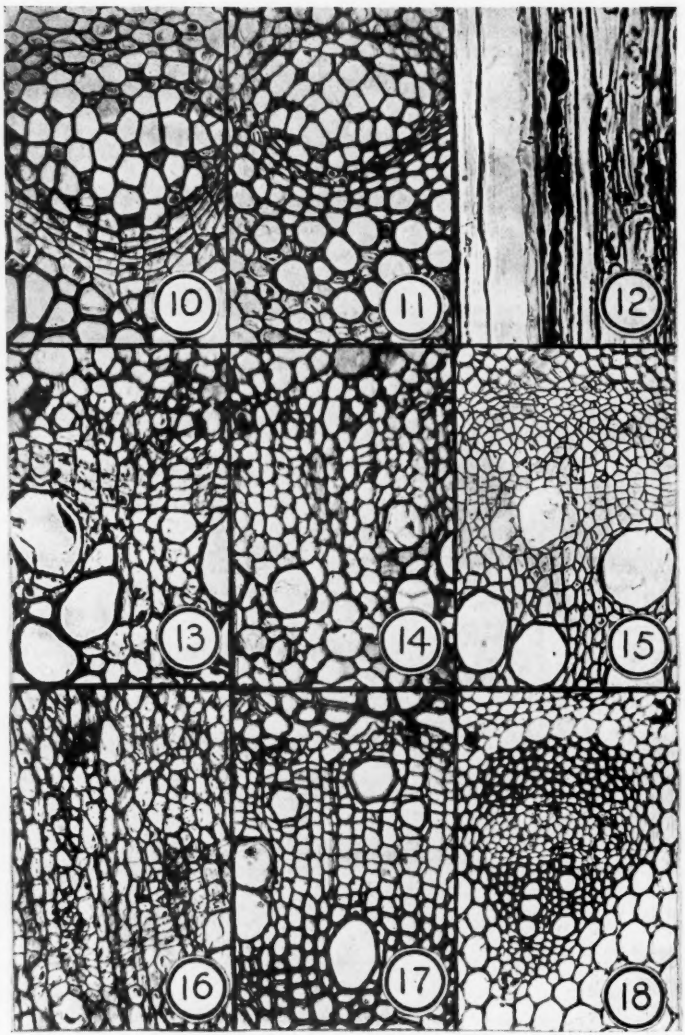
In the castor bean (figs. 5, 6), potato, marigold (figs. 8, 9), *Delphinium*, and a number of other plants the sieve tubes and companion cells are smaller in the flowering than in the non-flowering stem. In the majority of cases the walls of the cells of the phloem appear to be more thickened in the stems of the flowering plants.

Another characteristic of the stems of some flowering plants is the crushing of some of the phloem cells. A plant such as *Delphinium* (figs. 10, 11) shows sieve tubes and companion cells that have collapsed. Flowering stems presenting a similar condition are *Tropaeolum*, snapdragon, *Cleome*, and *Clematis*.

As the cambium ceases to be meristematic, phloem parenchyma rather than sieve tubes and companion cells is differentiated. This is well illustrated in stems of marigold, geranium, castor bean, and potato (figs. 13, 14).

In those plants in which callose was observed, as in geranium, alfalfa, and hemp (fig. 12), it is evident that the amount of callose on the sieve plates and sieve fields is greater in the stems of flowering plants than in the stems of non-flowering plants. The flowering stem of the ornamental lemon has a large amount of phloem tissue, but many more of these phloem cells have callose than have the cells in the non-flowering stem.

In a regenerating stem such as that of marigold, which has flowered but later became vegetative following pruning, two distinct regions of sieve tubes and companion cells are present (fig. 16). That is, the first group of sieve tubes and companion cells was formed before the plant blossomed, and during the flowering period



FIGS. 10–18.—Portions of sections of stems: Figs. 10, 11, *Delphinium ajacis* (10, non-flowering; 11, flowering). Fig. 12, *Cannabis sativa* (longitudinal section of flowering plant). Figs. 13, 14, *Solanum tuberosum* (13, non-flowering; 14, flowering). Figs. 15, 18, *Cosmos sulphureus* (15, non-flowering; 18, disbudded). Fig. 16, *Tagetes erecta* (flowered and later became vegetative following pruning). Fig. 17, *Fagopyrum esculentum* (below newly regenerated shoot which flowered immediately without period of vegetative growth).

phloem parenchyma was formed from the cambium. As the plant again became vegetative, the cambium was active and sieve tubes and cambium cells were again differentiated. In contrast to this condition, if the newly regenerating shoots immediately flower without a period of vegetative growth, as in *Fagopyrum esculentum* (fig. 17), there is practically no new phloem present. In such cases the new ring of tissue consists almost entirely of xylem cells.

Studies reported here are of samples of plants which are definitely vegetative or in advanced stages of flowering. One might ask if the anatomy which is characteristic of flowering stems is a result of flowering. It has been found from repeatedly disbudding plants such as *Cosmos* that the anatomy which is typical of flowering plants was present although they were prevented from flowering by disbudding (figs. 15, 18).

These observations raise the question: Do these details of the phloem suggest that an interrupted transport of elaborated materials causes blossoming in much the same way that girdling commonly results in flower production? The answer to this question may be partly solved by studies which are being made of samples collected during the period of transition from the non-blossoming to the blossom state. Sufficient progress has been made in the inspection of this material to permit of the preliminary statement that changes in the details of the anatomy of the phloem have been observed within a few days after the start of a change in environmental treatment, as short or long day, and as early as the appearance of blossom primordia.

Summary

From observations of the phloem of stems of a large number of plants, some structural differences between flowering and non-flowering plants were noted. There is a greater number of sieve tubes and companion cells in the non-flowering than in the flowering stem, and these cells are generally also larger in the vegetative stems. Some of the phloem cells in the flowering stems of many plants are crushed. The cambium in the flowering stem becomes less active and phloem parenchyma instead of sieve tubes and companion cells is formed. There is more callose in the phloem cells of the flowering

than in the non-flowering stem. These characteristics have been observed at the same time as the appearance of blossom primordia in several species.

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NITROGEN NUTRITION IN RELATION TO PHOTOPERIODISM IN XANTHIUM PENNSYLVANICUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 499

EDITH K. NEIDLE

(WITH ONE FIGURE)

Introduction

Many attempts have been made to correlate the effects of nutrient supply and the effects resulting when plants are subjected to various photoperiods. In general these attempts have shown that most plants which bloom at a short photoperiod cannot be induced to flower if grown continuously on long photoperiod, nor can plants which flower only on long photoperiod be induced to flower on short photoperiod, despite wide variations in the plane of nitrogen supply. MURNEEK (2) has recently reviewed the subject critically and added many experimental data.

Despite the fact that the induction of flowering in some plants seems to be more or less independent of the nitrogen supply in the nutrient medium, some of the other developmental phases of plants subjected to various photoperiods are definitely correlated with it. HAMNER (1) has pointed out that under the term flowering and fruiting condition, several developmental phases are included, such as: (1) the induction of the flowering condition, (2) the initiation of floral primordia, (3) the development of the floral primordia into mature flowers, (4) the development of fruits, (5) gametic union, and (6) the development of seeds. These phases are not all alike in the manner or extent to which they are maintained in relation to environmental factors, and should therefore be considered both independently and as they may be related to one another. It is with some of these points that this article deals.

The work was undertaken to determine what effect various planes of nitrogen supply may have upon the development of flowers and fruits in *Xanthium*, provided the plants are given a sufficient number of short photoperiods to bring about the induction of flowering and

the initiation of floral primordia. In general the plants were grown on long photoperiods of approximately 17.5 hours until they were about 10 inches tall. At this time all the plants were given an induction period of seven 8-hour photoperiods and the same number of 16-hour dark periods, with the exception of a few plants which were continued on long photoperiods to serve as controls. At the end of the induction period all plants subjected to the 8-hour photoperiod had initiated flower primordia. Half of them were then returned to long photoperiod and half continued on short photoperiod. The nitrogen supply to the plants was varied either by giving them a nutrient solution containing an abundance of nitrate nitrogen or one containing none. Two separate experiments were carried out and the results were approximately the same. For this reason only the results of one of them, from August 20 to January 19, are given here in detail.

Material and methods

Mature fruits of *Xanthium pennsylvanicum* were collected in the autumn of 1936 and stored in a dry place at room temperature. In August, 1937, the seeds were carefully removed from the burs and scratched slightly so as to rupture the seed coats. On August 20, six seeds were planted in each of sixty 2-gallon glazed crocks containing a medium grade of quartz sand. They were watered with distilled water for the first 7 days and thereafter a nutrient solution was supplied every third day. When germination had occurred, on August 25, 200 watt Mazda lamps, placed about 3 feet above the plants, were turned on each evening at 6:00 P.M. and off at midnight, giving the plants a total daily photoperiod of between 17 and 18 hours. During the very short days of the winter months the lamps were turned on at 4:30 P.M.

The following nutrient solutions were used:

PLUS N SOLUTION		MINUS N SOLUTION	
$NaNO_3$	638 p.p.m.	KH_2PO_4	212 p.p.m.
KH_2PO_4	212	$MgSO_4$	695
$MgSO_4$	695	$CaCl_2$	339
$CaCl_2$	339	$ZnCL_2$	1
$ZnCl_2$	1	$MnSO_4$	1
$MnSO_4$	1	$FeSO_4$	1
$FeSO_4$	1	$Na_2B_4O_2 \cdot 10\ H_2O$	1
$Na_2B_4O_2 \cdot 10\ H_2O$	1		

The first solution was supplied to all the plants until September 13. The plants were then approximately 6 inches tall and had two or three fully expanded leaves. On this date a division into two groups was made, one group of thirty pots was continued on the +nitrogen solution and the other group of thirty on the −nitrogen solution. On September 20 those plants which were receiving the −nitrogen solution were slightly smaller and somewhat more yellowish than the other plants; they showed no nitrates present on the basis of the di-phenylamine test.

On this date the number of plants was reduced to four per pot. Examination under the dissecting microscope showed no slightest indications of flower primordia. Two pots of plants from each treatment were so continued and subjected to long photoperiod. These served as controls. The remaining plants were transferred to a short photoperiod, to which they were subjected for 7 days. At the end of this induction period dissections showed that all the plants so treated had differentiated floral primordia.

At this time, therefore, four lots of plants were available for further experimentation: (1) those which had been grown continuously on long photoperiod and supplied with the + nitrogen solution; (2) those which had been grown continuously on the long photoperiod but which had been supplied with the −nitrogen solution subsequent to September 13; (3) those which had been given an induction period of seven short photoperiods and which had been continuously supplied with the +nitrogen solution; and (4) those which had been given an induction period but which had been supplied with the −nitrogen solution subsequent to September 13. Lots 1 and 2 were continued on the same treatment as previously given. Half of lot 3 and of lot 4 were continued at the short photoperiod and the other half of each lot returned to long photoperiod. A further subdivision was then made so that of the plants on long photoperiod half were placed on the +nitrogen solution and half on the −nitrogen solution. Thus there were ten groups of experimental plants. The treatments which the various groups of plants received are listed in figure 1. Those groups which were exposed to an induction period, and which developed flowers as a result of this treatment, are listed as groups II to IX. Thus certain of the plants were allowed to develop fruits on a short photoperiod and others on a long photoperiod.

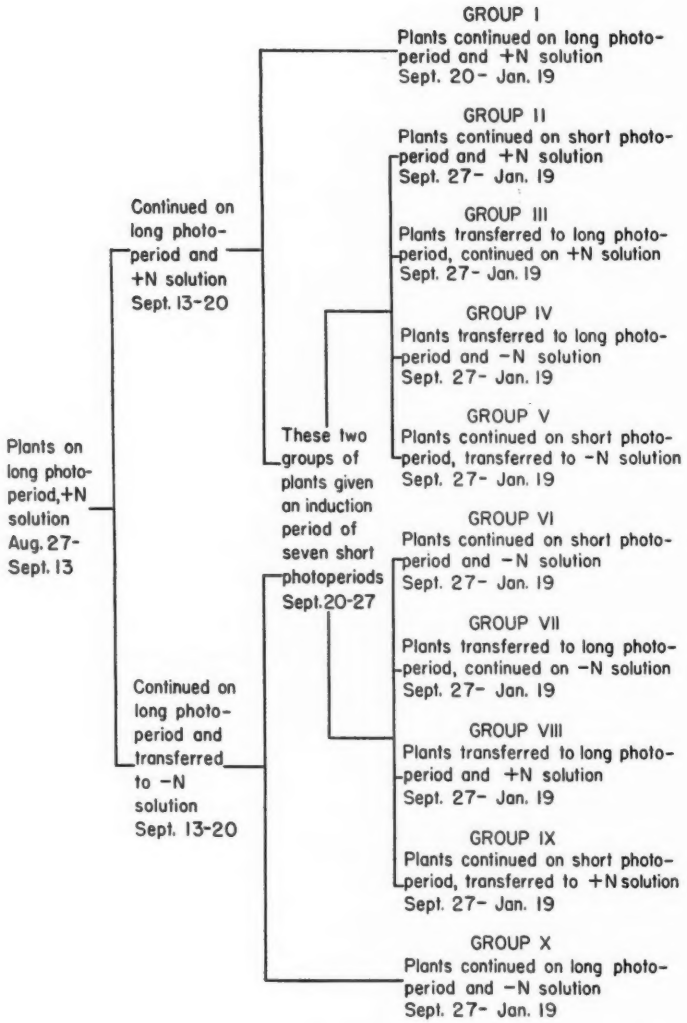
Plants on long photo-period,+N solution Aug. 27-Sept. 13
Continued on long photo-period and +N solution Sept. 13-20
Continued on long photo-period and transferred to −N solution Sept. 13-20
These two groups of plants given an induction period of seven short photoperiods Sept. 20-27
GROUP I
Plants continued on long photo-period and +N solution Sept. 20- Jan. 19
GROUP II
Plants continued on short photo-period and +N solution Sept. 27- Jan. 19
GROUP III
Plants transferred to long photo-period, continued on +N solution Sept. 27- Jan. 19
GROUP IV
Plants transferred to long photo-period and −N solution Sept. 27- Jan. 19
GROUP V
Plants continued on short photo-period, transferred to −N solution Sept. 27- Jan. 19
GROUP VI
Plants continued on short photo-period and −N solution Sept. 27- Jan. 19
GROUP VII
Plants transferred to long photo-period, continued on −N solution Sept. 27- Jan. 19
GROUP VIII
Plants transferred to long photo-period and +N solution Sept. 27- Jan. 19
GROUP IX
Plants continued on short photo-period, transferred to +N solution Sept. 27- Jan. 19
GROUP X
Plants continued on long photo-period and −N solution Sept. 27- Jan. 19

FIG. 1.—Diagram showing groups of plants used throughout the experiment

Results

In the tabulation and discussion of results the various lots of plants are referred to according to the listing in figure 1. Observations were made on the plants daily. The first plants to shed pollen did so on October 14, 17 days after the end of the 7-day induction period.

DEVELOPMENT OF STAMINATE INFLORESCENCES.—The rate of development of the staminate heads, the number produced, and the fertility of the pollen were all affected by both the length of photo-

TABLE 1

NUMBER OF PLANTS OF THE VARIOUS GROUPS SHEDDING POLLEN ON GIVEN DATES

GROUP	NO. OF PLANTS SHEDDING POLLEN				NO. OF PLANTS
	OCT. 14	OCT. 15	OCT. 19	OCT. 20	
II	7	10	25	25	27
III	0	0	5	19	19
IV	0	0	2	22	22
V	7	8	15	15	24
VI	0	1	13	13	25
VII	0	0	0	21	21
VIII	0	0	4	11	11
IX	1	3	20	20	22

period and the nitrogen supply after the induction period. The final results of the dates of pollen shedding by the plants of the various groups, the number of plants producing no staminate flowers, and the total number of staminate heads produced are shown in tables 1 and 2. The percentage of abortive pollen grains in the various groups is shown in table 3.

Those plants which were transferred to long photoperiod after the induction period developed flowers more slowly than those which were continued on short photoperiod. Although these differences are represented by a few days only, still these few days represent a considerable portion of the period of development, as for example, in the case of those from which pollen was shed 17 days after the end of the induction period. While those plants returned to long photo-

period developed flowers more slowly, nevertheless the staminate heads finally produced were larger and many more were produced than on those plants continued on short photoperiod. Every plant that was placed on long photoperiod subsequent to the induction

TABLE 2

PRODUCTION OF HEADS IN PLANTS OF SHORT PHOTOPERIOD

GROUP	NO. NOT PRODUCING STAMINATE HEADS	TOTAL NO. OF STAMINATE HEADS PRODUCED
II	2	80
V	9	34
VI	12	18
IX	2	54

TABLE 3

NUMBER OF ABORTIVE POLLEN GRAINS PER 100

GROUP	OCT. 28	OCT. 30	OCT. 30 (2ND COUNT)	OCT. 31	NOV. 1	NOV. 3	AVERAGE
II	84	59	80	70	64	0*	71.4
III	1	8	0†	4	9	1	4.6
IV	10	5	0†	8	3	9	7.0
V	38	0*	0*	9	18	26	22.5
VI	23	26	29	14	25	5	20.3
VII	79	2	0†	11	10	15	23.0
VIII	15	6	0†	4	15	10	10.0
IX	46	20	30	46	60	57	40.4

* Count was not possible because the whole flower cluster had dead anthers.
† No second count made.

period produced numerous staminate heads and these were produced over a long period of time, new ones developing several weeks after the first one had shed its pollen. On the other hand, the plants growing on short photoperiod produced relatively few staminate heads, some of them producing none, and those that were produced soon shed their pollen and no new ones developed. The amount of pollen abortion also was much greater in the plants continued on short photoperiod (table 3). These data are the result of frequent

observations of pollen grains prepared by the aceto-carmine smear technique and examined with the microscope.

Those plants receiving a low supply of nitrogen and returned to long photoperiod after the induction period more closely resembled the plants on short photoperiod treatment than did the plants which were continued on a high nitrogen supply and returned to long photoperiod. In contrast to this, those plants on short photoperiod with a high nitrogen supply more closely resembled the plants on long photoperiod (tables 1–4).

TABLE 4

SIZE OF ANTHERS OF VARIOUS GROUPS GROWN ON DIFFERENT TREATMENTS

Group	Measurements in mm.			
	Length (base of pollen area to tip)	Width	Length of pollen bearing area	Width of pollen bearing area
II	0.891	0.286	0.542	0.116
III	1.085	0.333	0.790	0.115
VI	0.910	0.298	0.581	0.146
VII	0.910	0.340	0.705	0.186

Development of pistillate heads.—The effect of nitrogen supply upon the development of pistillate flowers was somewhat similar to that upon staminate flowers. In every case the more abundant the supply of nitrogen the greater the number of carpellate flowers. This was true under both the long and the short photoperiod conditions. Plants on short photoperiod developed pistillate flowers much more rapidly and were receptive to pollen 2 weeks before any flowers from the plants on long photoperiod. Data for the carpellate flowers counted on October 21 are given in table 5.

Those plants which were returned to long photoperiod after the induction period produced relatively few pistillate flowers and relatively few burs (table 6). The total number of burs produced by the plants on short photoperiod was far greater than the total number produced by the plants on long photoperiod. Many of the fruits

from plants on short photoperiod contained no seeds. In group VIII, although 711 burs were produced, only 132 contained seeds, and many burs developed parthenocarpically. Perhaps one of the reasons for so many of the short photoperiod fruits developing without seeds might be correlated with the fact that a number of carpellate flowers were receptive for pollination before any pollen was available. That

TABLE 5

NUMBER OF PISTILLATE FLOWERS PRODUCED IN INDIVIDUAL PLANTS BY OCTOBER 21*

GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI	GROUP VII	GROUP VIII	GROUP IX
24	5	3	15	17	1	0	24
23	6	2	25	16	0	3	15
24	4	3	13	6	1	3	12
21	5	3	15	12	0	2	24
27	0	5	20	11	3	5	13
15	5	2	11	7	3	1	29
18	6	3	15	8	1	0	15
27	0	4	21	9	3	4	16
16	5	3	8	5	2	0	13
33	2	3	23	6	1	0	20
19	4	4	12	6	1	1	27
20	0	.....	28	7	0	5	25
28	7	.....	14	9	4	2	.....
33	3	.....	22	17	5	3	.....
36	7	.....	.....	17	1	0	.....
14	7	.....	.....	20	.....	4	.....
	AVERAGE PER PLANT						
21.4	4.1	3.1	15.2	10.8	1.7	2.0	16.4

* After this date the plants growing on long photoperiod (groups III, IV, VII, VIII) produced more flowers.

is, the pistillate flowers on the short photoperiod treatment developed more rapidly than the staminate flowers, and the styles and stigmas withered away before pollination had taken place.

The weight of the burs produced on long photoperiod was approximately twice as great as those on the short photoperiod (tables 7, 8). The weight of the seeds of the various groups was also greatly affected.

In every case the plants grown on short photoperiod produced only one crop of burs. Shortly after maturation of these, the plants

under this treatment died. Those plants on the long photoperiod having little nitrogen available (groups IV and VII) were comparable in this respect with short photoperiod plants in that they produced only one crop of burs. While an abundant supply of nitrogen in its effect upon the number of burs developed is somewhat similar to the effect of long photoperiod, in its effect upon the weight of seeds it is in direct contrast to the effect of length of photoperiod. Thus the burs of the plants on the +nitrogen treatment were always lighter than the burs on the −nitrogen treatment, whereas the individual seeds of the former were always heavier than those of the latter.

TABLE 6

NUMBER OF BURS PRODUCED BY GROUPS OF PLANTS OF THE VARIOUS TREATMENTS

Group	Number of burs					
	Total	Average per plant	No. with seed	Av. with seed per plant	No. without seed	No. in 2nd crop
II	521	21.7	181	7.1	340	0
III	201	10.5	200	10.5	1	25
IV	158	7.0	156	7.0	2	0
V	335	15.5	165	7.1	170	0
VI	258	10.2	47	1.9	211	0
VII	103	5.0	99	4.7	4	0
VIII	96	8.7	96	8.7	0	5
IX	711	32.3	132	6.0	579	0

The length of the photoperiod had more effect than the nitrogen supply in determining the total heights of the plants, as shown in table 8. The plants grown on long photoperiod were in general about 5 inches taller than the plants grown on short photoperiod. The plants returned to long photoperiod did not die after producing one crop of fruit, but continued growing slowly for a long time. Certain plants were placed under continuous illumination on December 15 and were continued on this treatment for more than 7 months. During this entire period these plants continued to grow and produce additional flowers and fruits. Many of the bracts in the staminate inflorescence somewhat resembled green foliage leaves. Certain of the staminate flowers possessed ten or more stamens in a single

flower. In certain cases, after a staminate head had been partially formed, the apical internodes of the axis of the staminate inflorescence continued elongation, producing one or more long internodes,

TABLE 7

NUMBER AND WEIGHT OF BURS PRODUCED BY PLANTS OF THE VARIOUS GROUPS

GROUP	NO. OF BURS	WEIGHT OF BURS (GM.)	AVERAGE WEIGHT OF BURS (GM.)
II	198	21.0	0.106
III			
1st crop	175	35.9	.205
2nd crop	25	4.7	.188
IV	156	33.5	.214
V	150	17.0	.113
VI	49	6.5	.132
VII	99	21.5	.217
VIII			
1st crop	144	31.2	.216
2nd crop	6	1.2	.200
IX	120	15.5	0.129

TABLE 8

WEIGHT OF SEEDS PRODUCED BY PLANTS OF THE VARIOUS TREATMENTS AND THEIR AVERAGE HEIGHT

GROUP	TREATMENT	WEIGHT OF 10 SEEDS (GM.)	AVERAGE HEIGHT (INCHES)
	Short photoperiod		
II	$+NO_3$	0.790	$13\frac{3}{4}$
V	$+$ and $-NO_3$	.683	12
IX	$-$ and $+NO_3$	.780	$13\frac{1}{2}$
VI	$-NO_3$	.723	$11\frac{1}{2}$
	Long photoperiod		
III	$+NO_3$	.912	$19\frac{1}{2}$
IV	$+$ and $-NO_3$	.690	$17\frac{1}{2}$
VIII	$-$ and $+NO_3$	.905	18
VII	$-NO_3$	0.883	$14\frac{3}{4}$

and then a new staminate head would be formed. As a result of the intermittent vegetative growth and flowering, some of the plants on long photoperiod increased in height manyfold during the months following the 7-day induction period. The vegetative growth of the

plants on long photoperiod was made only by those plants which received an abundant supply of nitrogen. Those plants which received a limited supply, after the induction period, produced one crop of fruit and soon died. No matter how abundant the supply of nitrogen to the plants on short photoperiod, they did not grow vegetatively to any marked degree, whereas the nitrogen supply to the plants returned to long photoperiod had a very definite effect.

In comparing plants which had been subjected to an induction period with plants which had not been so treated but which had been grown continuously on long photoperiod, and consequently were vegetative, certain differences were observed. The controls growing on long photoperiod and with an abundant supply of nitrogen produced a great amount of vegetative growth over a period of several months. These plants branched very little, but attained a height of more than 5 feet. The plants on long photoperiod, which had received an induction period of seven short photoperiods and which also had received an abundant supply of nitrogen, did not produce as much growth but branched more profusely. The control plants continued on long photoperiod but transferred to a minus solution on September 13 produced relatively little growth in height. In comparing these plants with those grown on a −nitrogen solution and a long photoperiod and which had received an induction period, the control plants were smaller and had less dry weight. Microscopic examination of the stems showed that the control plants were smaller in diameter and contained many starch grains and calcium oxalate crystals. The plants of group VI, on the other hand, were larger in diameter and contained no starch grains or calcium oxalate crystals.

Summary

1. Plants of *Xanthium pennsylvanicum* were grown in sand cultures containing either an abundance of nitrogen or very little. They were first grown on long photoperiod. Then all were given an induction period of seven short photoperiods and then one-half were returned to long photoperiod, the other half continuing on short. The plants continued on short photoperiod and receiving an abundant supply of nitrogen bloomed first; those of the same type at a low supply of nitrogen bloomed next; those on long photoperiod and

high nitrogen supply were the third to bloom; those on long photoperiod and low nitrogen were the last to bloom.

2. Of the short day plants, those receiving an abundant supply of nitrogen produced the greatest number of staminate heads. All the plants on long photoperiod produced more staminate flower clusters than the plants on short photoperiod. The percentage of abortive pollen grains was greatest on the plants on short photoperiod. In the latter a low plane of nitrogen supply resulted in the greatest number of defective pollen grains, and in the plants on long photoperiod a high supply of nitrogen resulted in the same effect.

3. There were more fruits per average plant which received a short photoperiod than a long one. Whether the photoperiod was long or short, those given an abundant supply of nitrogen had many more burs than those given a low supply. More seeds were found on plants on long photoperiod because many of them on short photoperiod had empty burs, many of the latter developing parthenocarpically.

4. Plants grown on a long photoperiod following an induction period with a low supply of nitrogen bore fruits and were larger than control plants grown on a similar long photoperiod with no induction period but with a similar low nitrogen supply. The latter bore no fruits.

This investigation was carried out under the direction of Dr. K. C. HAMNER, Department of Botany, University of Chicago.

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STUDIES OF PROTOPLASMIC STRUCTURE IN SPIROGYRA. IV. EFFECTS OF TEMPERATURE ON PROTOPLASMIC ELASTICITY[1]

HENRY T. NORTHEN

(WITH ONE FIGURE)

Introduction

Because the effect of temperature is not entirely an effect on the actual chemical processes, as evidenced by inconstancies of Q_{10}'s and lowerings of optimum temperatures with increases of exposure, it is conceivable that temperature alters the structure of protoplasm and that such alterations influence the character and rate of various processes.

The data presented in this paper indicate that as the temperature is raised from 1° to 38° C., the elasticity of the protoplasm in cells of *Spirogyra* decreases. Presumably changes in elasticity involve alterations in protoplasmic structure. The possible nature of such alterations is discussed and an attempt has been made to correlate alterations in structure with some biological phenomena.

The elasticity of the protoplasm was considered decreased if increases in temperature lowered the value of c_0 in the equation $V = k(c - c_0)$, which approximately governs the rate of chloroplastic movement in cells of *Spirogyra* in response to centrifugal accelerations (7). In the equation, V is the velocity of chloroplastic movement, k a constant, c the centrifugal acceleration used, and c_0 the initial starting acceleration at which or below which the chloroplasts will not move regardless of how long the cells are centrifuged.

Method

Filaments of *Spirogyra* were laid between two strips of moist cotton, and these cotton wads were then placed in centrifuge tubes.

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 167. This investigation was supported partially by a grant from the Research Committee of the University of Wyoming.

The tubes were immersed for the desired time in a constant temperature bath (durations of immersion and temperatures used are recorded in table 1). Next the tubes were removed and the filaments

TABLE 1

EFFECT OF TEMPERATURE ON VALUE OF c_0 IN EQUATION $V = k(c - c_0)$

EXPERIMENT NO.	TEMPERATURE (°C.) AT WHICH FILAMENTS WERE MAINTAINED	MINUTES IMMERSED	CENTRIFUGAL ACCELERATION × GRAVITY	MINUTES CENTRIFUGED	PERCENTAGES OF FILAMENTS IN WHICH CHLOROPLASTS WERE DISPLACED
1	1.0 5.0 6.5 8.5 11.5	6	1062.0	0.25	1 25 37 35 61
2	1.0 5.0 6.5 8.5 11.5 15.0 19.0 23.0	6	680.0	0.50	0 4 11 22 35 61 65 93
3	23.0 28.0	6	170.0	2.00	8 81
4	23.0 28.0 33.0	6	75.5	4.50	0 4 76
5	33.0 38.0 43.0	6 4 3	42.5	8.00	5 85 44
6	43.0 43.0 43.0 43.0	3 4 6 12	680.0	0.50	95 62 19 6

were centrifuged (see table 1 for accelerations used) in a chamber which was at approximately the same temperature as that of the bath. The data were obtained by determining the percentages of filaments in the cells of which the chloroplasts had been displaced (chloroplasts moved to the end of the cell). For each experimental group about 300 filaments were used. Previous experiments have

demonstrated that at 43° C. about 2 minutes is required for the temperature equilibrium to be reached in the cotton wads.

Data

Consider the change in elasticity, as evidenced by a decrease in the value of c_0, between temperatures of 1° and 38° C. It will be noted (experiment 1) that at 1° C. the chloroplasts were displaced in only 1 per cent of the filaments. This means that in 99 per cent of them the value of c_0 was equal to or greater than 1062 × gravity, and accordingly the chloroplasts would not move in response to a centrifugal acceleration of 1062 because:

$$V = k\,(1062 - 1062) = 0 \text{ or } V = k\,(1062 - > 1062) = < 0.$$

On the other hand, at 38° C. (experiment 5) c_0 was less than 42.5 × gravity for the 85 per cent in which the chloroplasts were displaced and equal to or greater than 42.5 × gravity for the 15 per cent of the filaments in which the chloroplasts were not displaced. According to theory, for 85 per cent of the filaments,

$$V = k(42.5 - < 42.5) = > 0.$$

Table 1 also shows that up to 38° C. as the temperature increased the effective acceleration decreased. This means that between temperatures of 1° and 38° C. the value of c_0 decreased, indicating that as the temperature increased the protoplasmic elasticity decreased. At 23° C. the value of c_0 was less than 170 × gravity for only 8 per cent of the filaments (experiment 3), but at 43° C. c_0 was less than 42.5 × gravity for 44 per cent of the filaments (experiment 5). This indicates that following 3 minutes' immersion at 43° C. the elasticity of the protoplasm in many cells was less than in most cells maintained at 23° C. Experiment 6 demonstrates that at 43° C. exposures greater than 3 minutes caused a coagulation of the protoplasm. Perhaps in the 56 per cent (100 − 44 per cent) of the filaments in which no movement of the chloroplasts was observed after 3 minutes' immersion (experiment 5) the protoplasm had coagulated during the 20 seconds required to transfer the tubes to the centrifuge or during the first minute of centrifugation before a noticeable displacement of

the chloroplasts had occurred. The 44 per cent in which the chloroplasts were displaced certainly indicate that a decrease in elasticity preceded coagulation. What occurred in 44 per cent of the filaments undoubtedly occurred in all, but the time required to transfer the tubes and to centrifuge the filaments militated against an actual determination of this.

To summarize, the data indicate that as the temperature was raised from 1° to 38° C., the elasticity of the protoplasm in cells of *Spirogyra* decreased. At 43° C., coagulation was preceded by a decrease in elasticity.

Discussion

Elastic fluids, such as the protoplasm in which the chloroplasts of *Spirogyra* are imbedded, are presumably made up of interlaced or variously united linear molecules.

FREY-WYSSLING (4) has deduced that protoplasm must have such a structure. He thinks that the basic or acidic end groups of long protein molecules combine with organic or inorganic ions, and that certain end groups of side chains combine with either hydrophilic or lipophilic molecules to produce a molecular complex. The molecular complexes combine with one another to produce a structural network. According to FREY-WYSSLING, the molecular complexes can unite through homopolar cohesions between neighboring alkyl groups, through heteropolar cohesions between neighboring hydroxyl groups, through heteropolar valence combinations between basic and acidic groups, or through valence combinations between reduced alkyl or sulph-hydryl groups.

Figure 1 shows the possible arrangements of protein and lipoid molecules. Solid lines represent proteins and dotted lines lipoids. Carbohydrates have not been represented but they may be combined with proteins or exist in solution in the interstices. Salts may be combined with the proteins, lipoids, or carbohydrates, or exist in solution in the interstices. The structure as represented in the diagram accounts for the observed elasticity. In order to move an object (such as a chloroplast) suspended in such a fluid, a definite shearing stress, c_0, must be exceeded.

Temperature may alter the structure represented in the diagram

in various ways. It may decrease or break some cohesions. FREY-WYSSLING states that the homopolar cohesions between alkyl groups are very sensitive to temperature. Temperature may break some valence combinations or it may alter the shape of some molecules, particularly protein molecules. The separation of network constituents by a rise in temperature will loosen the structure and concomitantly decrease the elasticity of the protoplasm, which is in accordance with the data presented.

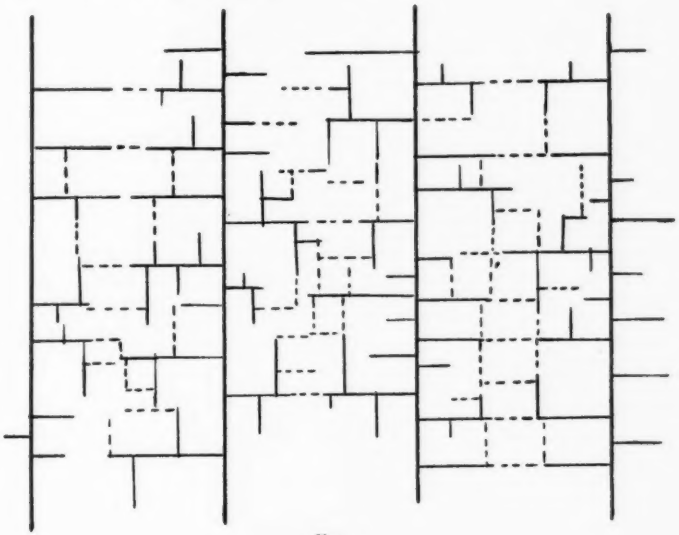

FIG. 1

The release of molecules from the network or the loosening of the network may help to explain the breaking of rest periods in plants. It is significant that warm baths, injury (**10**), and ether in certain concentrations and exposures (**8**) decrease protoplasmic elasticity, and that all of these treatments break rest periods.

The following deals with speculations on the nature of the plasma membrane. It may have a structure comparable with that of the diagram but perhaps more compact. Such a membrane may be considered a mosaic of lipoids and proteins, as BROOKS (**2**) has postulated. It is reasonable to think that such a membrane would be

affected by temperature as would the protoplasm in which the chloroplasts of *Spirogyra* are imbedded. If the preceding ideas are correct, an increase in temperature would loosen the network, increase the size of the pores, and increase the rate of permeability of at least those substances whose entrance is governed by pore size. It is known that, within limits, the higher the temperature the greater the rate of permeability (1). Many fat solvents enter cells rapidly. NORTHEN (8) has demonstrated that many fat solvent anesthetics in certain concentrations and exposures decrease protoplasmic elasticity in *Spirogyra*. The change in structure which the fat solvents induce may be responsible in part for the rapidity with which they enter. The present concept of the membrane also explains why such anesthetics should increase the rate of permeability of substances other than fat solvents (5). As previously mentioned, fat solvent anesthetics decrease elasticity and loosen the protoplasmic network. The concept also explains why lethal temperatures destroy the selective permeability of the membrane. Presumably such temperatures destroy the protoplasmic network.

The mechanism of heat death will be developed from the fact that a decrease in elasticity precedes coagulation. The suggested mechanism is a combination of the lipoid liberation and protein coagulation theories as discussed by BĚLEHRÁDEK. The first effect of lethal temperatures is to liberate the lipoids from their combinations with proteins in the protoplasmic network. This liberation loosens the structure and accounts for the observed decrease in protoplasmic elasticity. The liberated lipoid molecules fuse to form droplets, and the protein molecules, no longer separated in the network, fuse to form a coagulum. The heat itself may cause the coagulation; or the heat working synergistically with the salts present in the interstices or liberated from the network may cause the protein coagulation. That salts may be liberated has been demonstrated by MAZIA and CLARK (6). They found that a temperature of 40 °C. releases free calcium in *Elodea* cells. When the network is destroyed the solutions in the interstices form vacuoles, and vacuolization is known to accompany heat death (1).

According to the preceding hypothesis, conditions or substances

which decrease elasticity (loosen the structure) should accelerate heat death. As previously mentioned, anesthetics and mechanical injury decrease elasticity. The data of FEICHTINGER (3) suggest that mild radium treatment decreases protoplasmic elasticity. BĚLEHRÁDEK reports that anesthetics, mechanical injury, and radiations hasten heat death.

Conversely conditions which increase elasticity (tighten the network) should increase the resistance to heat. NORTHEN (9) has found that the elasticity of the protoplasm in cells of rye coleoptiles increases during drought. BĚLEHRÁDEK reports that the lower the water content of cells the greater the resistance to heat.

The hypothesis may also explain why pseudopods are withdrawn when certain organisms are exposed to high temperatures (1). Surface tension and the elasticity of the surface of such organisms tend to pull the pseudopods into the main body so that the organism will assume the shape of least free energy. Under normal conditions the pseudopods are maintained because of their structural network. When this network is destroyed by high temperature, no support remains and surface tension withdraws the pseudopods.

Summary

1. Filaments of *Spirogyra* were immersed for different periods in water maintained at various temperatures, and were centrifuged at these temperatures with different accelerations.

2. The data indicate that between 1° and 38° C. an increase in temperature lowered the effective centrifugal acceleration. For example, the low acceleration of 42.5 × gravity caused displacements of the chloroplasts in cells of many filaments maintained at 43° C. for 3 minutes and at 38° C. for 6 minutes, but in practically none of those maintained at 33° C. An acceleration of 75.5 × gravity caused significant displacements in cells maintained at 33° C. but not in cells maintained at 28° or at 23° C.

3. Between 1° and 38° C. an increase in temperature decreased the value of c_o in the equation $V = k(c - c_o)$, which approximately governs the rate with which the chloroplasts in cells of *Spirogyra*

move in response to centrifugal accelerations. A decrease in c_0 was interpreted as a decrease in protoplasmic elasticity.

4. At 43° C. coagulation is preceded by a decrease in protoplasmic elasticity.

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GROWTH AND METABOLISM OF BEAN CUTTINGS SUBSEQUENT TO ROOTING WITH INDOLEACETIC ACID

JOHN W. MITCHELL[1] AND NEIL W. STUART[2]

(WITH SIX FIGURES)

Introduction

Several investigations (1, 2, 4, 6, 10) have demonstrated that the application of beta-indoleacetic acid to cuttings or entire plants affects their metabolism and rate of transfer of carbohydrate and nitrogenous compounds. In a recent study (11) it was shown that the rate of transfer of these compounds, from the top downward to the hypocotyls where roots were initiated, was definitely accelerated by the application of indoleacetic acid to the bases of bean cuttings. The treated cuttings responded by rapid swelling of the hypocotyls, increased root production, and a temporary suppression of top growth. The investigation referred to was limited to a study of the responses that occurred during a period of 120 hours after treatment, the time necessary for the initiation and emergence of roots.

In connection with this earlier work, the present experiments were undertaken to compare the growth and the carbohydrate and nitrogen metabolism of treated and untreated cuttings subsequent to the time of root emergence. Several thousand cuttings were treated with indoleacetic acid solutions of two different concentrations and then grown, together with untreated cuttings, for 2 weeks in a greenhouse. The fresh weight, dry weight, and the carbohydrate and nitrogen content of various parts of the treated and untreated plants were compared at 2 day intervals during a 10 day period following root emergence.

Investigation

MATERIALS AND TREATMENT.—Approximately 8000 seedlings of kidney beans, *Phaseolus vulgaris*, were grown in soil in a greenhouse

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on two adjacent benches. On the tenth day following planting of the seeds, the plants were 5–7 inches in height, the primary leaves had expanded to approximately two-thirds of their final size, but the second internodes had not elongated appreciably and the trifoliate leaves were not expanded. The cotyledons had decreased in size, and although measurements were not made, it appeared that most of the food materials they originally contained had been translocated. During this 10 day period the temperature in the greenhouse varied between 70° and 85° F. and the light intensity from 2000 to 5000 foot candles.

Each bench was marked into two equal parts, and approximately 5200 cuttings were made October 14 by cutting selected plants through the hypocotyls a measured distance of 2 inches below the cotyledons, and collecting plants in equal numbers from each section of the benches. The cotyledons were removed; and to prevent wilting, groups of approximately 250 cuttings were immediately placed in galvanized iron treating trays containing tap water to a depth of approximately 1 inch. The cuttings were divided into three groups of equal numbers and two of these groups were treated for 3 hours by immersing the hypocotyls in solutions of indoleacetic acid dissolved in tap water. The first group was treated with a 0.002 per cent solution; this was known to be near the lowest concentration effective in stimulating root production. The second group was treated with a 0.01 per cent solution which was known to be strongly effective in stimulating root production. The remaining group was treated with tap water for an equal interval of time and used as a control.

The indoleacetic acid solutions were prepared by dissolving the required amount of acid in 10 cc. of 95 per cent alcohol. The alcoholic solutions were then added to 9 liters of water, and placed in the treating trays where the cuttings stood, during the 3 hour period of treatment, at room temperature in diffused light. They were then planted to a uniform depth of 2 inches in unsterilized quartz sand contained in 4 inch clay pots. Four cuttings were placed in each pot, and the sand was carefully washed around the hypocotyl. For the next 5 days the humidity of the surrounding air was maintained between 65 and 85 per cent by inclosing the sides and top of each bench with glass. The sand was kept moistened with tap water and the

plants were protected from intense light, the maximum intensity being approximately 1000–2000 foot candles during the first 5 days, and 5000–8000 foot candles during the remainder of the experiment, except on the ninth and tenth days after treatment when clouds re-

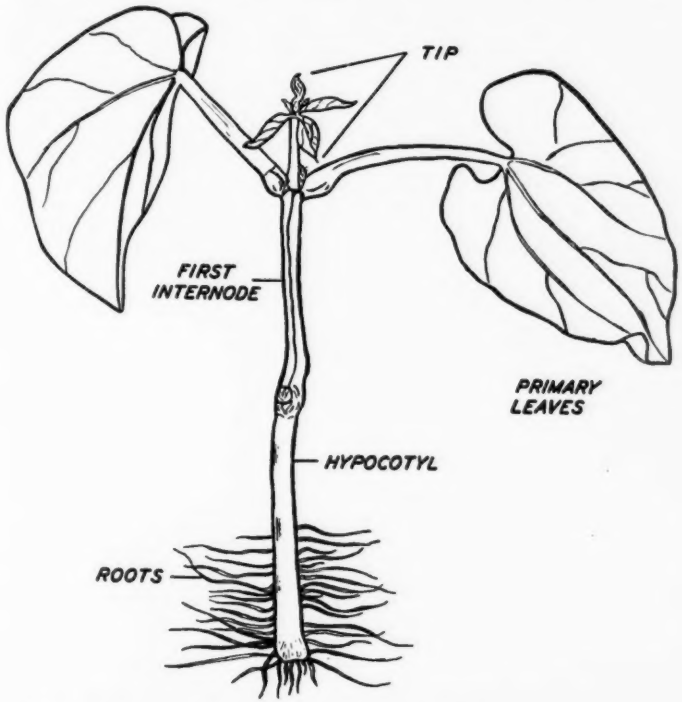

FIG. 1.—Parts into which plants were divided for weight determinations and chemical analyses. Diagram shows appearance of cutting 7 days after treatment with 0.01 per cent indoleacetic acid.

duced the intensity. The temperature generally varied between 70° and 85° F. but on one occasion reached 90° F. for a short period of time. The pots were arranged on two parallel adjacent benches that were marked into eight sections of the same size. An equal number of plants of each treatment were evenly distributed throughout each section.

On the fifth day following treatment, roots had emerged from the hypocotyls and the first harvest was made. Individual samples were systematically collected from pots in corresponding positions in each of the eight sections. The sand was carefully washed free from the roots, the plants divided into five parts (fig. 1), and the fresh weight of each part recorded. The original eight samples were then combined so that there were quadruplicate samples of each of the five parts. The tissue was dried in a well ventilated oven at 80° C. and the dry weights recorded. The samples were again combined, so that duplicate samples of each part were used for chemical analyses.

TABLE 1

ANALYSIS OF VARIANCE OF DRY WEIGHT DATA FROM TABLE 3

Source of variation	Degrees of freedom	Mean square for parts			
		Hypocotyls	First internodes	Tips	Roots†
Total	71	0.0634	0.0735	19.847	0.2984
Treatment	2	0.6514*	0.1688*	3.370*	0.8180*
Plot	3	0.0213	0.0065	1.688*	0.0748
Time	5	0.3722*	0.8842*	277.140*	3.6126*
Remainder (error)	61	0.0209	0.0072	0.1918	0.0259

* Significantly greater than error of variance by odds of more than 99 to 1.

† Degrees of freedom: total 59; time 4; and remainder 50.

One of these samples was therefore made up of plants from one bench and one of plants from the other.

Subsequent harvests were made on every second day for a period of 10 days. During this interval the unharvested plants were supplied, on days between sampling, with a complete nutrient solution (8). The dry weight data for hypocotyls, first internodes, tips and roots were analyzed statistically for variance (table 1), and the difference in grams of dry matter per mean sample necessary for significance was determined (table 3).

CHEMICAL METHODS.—Two separate samples, obtained as previously described, were ground to pass a 40-mesh sieve and analyzed. The moisture content was determined by drying duplicate portions at 100° C. Sugars were extracted with 80 per cent alcohol in Soxhlets. The reducing power of the deleaded extracts was determined

before and after inversion with invertase by the Munson and Walker method, followed by titration with potassium permanganate. Direct reducing sugars were expressed as glucose and total sugars as invert sugar. Sucrose was calculated by multiplying the difference between total sugars and reducing sugars (as invert) by 0.95.

Total nitrogen was determined by the reduced iron modification of the Gunning method (9), to include nitrates. Analysis was made for the insoluble nitrogen in the residue remaining after extraction of the sugars by the Gunning method, and a correction made for the extracted weight of the samples.

TABLE 2

EFFECT OF INDOLEACETIC ACID TREATMENT ON NUMBER AND DRY WEIGHT OF ROOTS PRODUCED BY BEAN CUTTINGS. DRY WEIGHTS AND ROOT COUNTS REPRESENT 200 PLANTS. THE NUMBER OF ROOTS ON 50 PLANTS OF EACH TREATMENT WERE COUNTED

TREATMENT	DRY WEIGHT 7TH DAY (GM.)	DRY WEIGHT 15TH DAY (GM.)	INCREASE IN DRY WEIGHT (GM.)	AVERAGE NUMBER OF ROOTS 15TH DAY	AVERAGE DRY WEIGHT PER ROOT 15TH DAY (MG.)
Control.....	1.2	6.7	5.5	9800	0.68
0.002%.....	1.3	6.3	5.0	9700	0.65
0.01%.....	2.7	7.1	4.4	15960	0.44

Results

GROWTH RESPONSES.—The hypocotyls of cuttings treated with a 0.01 per cent solution of indoleacetic acid increased in size more rapidly during the first week following treatment than did those treated with 0.002 per cent or the controls. Most of the roots emerged on the fifth day. Relatively fewer roots were produced by the control and weaker treatment, and these were clustered toward the lower end of the hypocotyls, while plants treated with the stronger solution produced roots in great numbers (table 2), which in general were developed over a considerable portion of the hypocotyls. Although plants treated with the 0.01 per cent solution produced more roots, their average size appeared to be less than that of either those of controls or those of the plants that were treated with the less concentrated solution.

There was an evident increase in the height and leaf area of the controls and also the plants treated with 0.002 per cent acid solution during the 5 days immediately following treatment. Those treated with the more concentrated solution, on the other hand, showed no appreciable increase in height or leaf area until the seventh day. On the eleventh day following treatment, control plants were approximately 8–10 inches in height, those treated with 0.002 per cent solution 6–8 inches, and those treated with 0.01 per cent solution 4–6 inches above the ground level. This difference became less apparent toward the end of the experiment, and on the fifteenth day there was little difference in the height or general appearance of the plants in all three treatments.

The dry weight of the roots of plants treated with 0.002 per cent acid increased by approximately the same amount as those of controls during the experiment, whereas those of plants treated with a 0.01 per cent solution contained 130 per cent more dry matter than did those of untreated plants, 7 days after treatment (table 2). The roots of untreated plants grew more vigorously toward the latter part of the experiment, however, and at the time of the final harvest the difference in dry weight of the total number of roots on treated and untreated plants was only approximately 6 per cent (fig. 2, table 2). The average dry weight of individual roots of control plants, and also of those treated with 0.002 per cent acid, was approximately 52 per cent greater than those of plants treated with the more concentrated solution.

The dry weight of the hypocotyls of plants treated with a 0.01 per cent solution was significantly greater than that of untreated plants during the early part of the experiment, but this difference also decreased until the amount of solid matter in hypocotyls of treated and untreated plants was not significantly different at the time of the final harvest (table 3, fig. 2). The amount of solid matter in the first internodes of treated plants increased more rapidly than that of untreated plants only during the first 5 days following treatment (fig. 3). The dry weight of the primary leaves decreased approximately the same amount in all treatments during the experiment, and this decrease was most rapid during the 5 days immediately following treatment (fig. 3). The dry weight of the tips

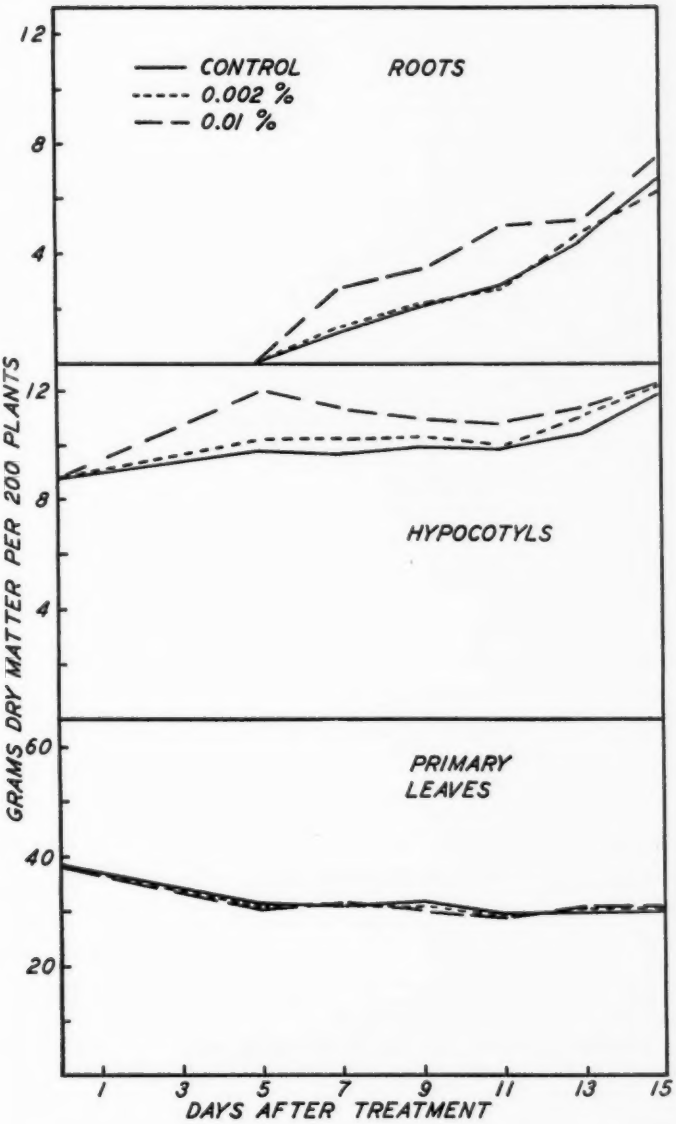

FIG. 2.—Dry weights of various parts of cuttings treated with indoleacetic acid solutions as compared with those of untreated plants.

of control plants increased more rapidly and became significantly greater than that of treated plants by the ninth day following treatment (fig. 3), and this difference remained significant throughout the remainder of the experiment.

TABLE 3

DRY WEIGHTS* OF VARIOUS PARTS OF BEAN PLANTS GROWN IN QUADRUPLICATE PLOTS AND TREATED WITH 0.002 PER CENT (WEAK) AND 0.01 PER CENT (STRONG) SOLUTIONS OF INDOLEACETIC ACID, AS COMPARED WITH WEIGHTS OF SIMILAR PARTS OF UNTREATED PLANTS. FIGURES REPRESENT AVERAGE WEIGHT PER SAMPLE (56 PLANTS) PER PLOT

DAYS AFTER TREATMENT	CONTROL	WEAK	STRONG	CONTROL	WEAK	STRONG	CONTROL	WEAK	STRONG
	ROOTS			HYPOCOTYLS			FIRST INTERNODES		
Initial check	.....	.....	.....	2.47	.....	.....	1.40	.....	.....
5th	.....	.....	.....	2.75	2.86	3.37†	1.96	1.88	1.80†
7th	0.333	0.351	.762†	2.71	2.86	3.19†	1.94	1.94	1.78†
9th	0.595	0.599	.949†	2.78	2.90	3.08†	2.21	2.12	2.01†
11th	0.81	0.805	1.39†	2.76	2.78	3.04†	2.12	2.13	1.99†
13th	1.24	1.31	1.49†	2.91	3.09	3.09	2.38	2.32	2.26†
15th	1.86	1.77	1.99	3.33	3.32	3.40	2.66	2.63	2.47†
	LEAVES			TIPS			TOTAL		
Initial check	10.71	.....	.....	0.403	.....	.....	14.983	.....	.....
5th	8.91	8.55	8.47	0.90	0.76	0.55	14.52	14.05	14.19
7th	8.68	8.76	8.75	1.64	1.54	1.13	15.303	15.451	15.612
9th	9.00	8.72	8.63	3.54	3.20	2.65†	18.125	17.539	17.319
11th	8.27	8.20	8.37	6.10	5.87	5.20†	20.06	19.785	19.99
13th	8.30	8.42	8.62	9.35	9.22	8.60†	24.18	24.36	24.06
15th	8.42	8.45	8.50	13.55	13.22	12.57†	29.82	29.39	28.93

* Differences between weights required for significance: hypocotyls 0.20; first internodes 0.12; tips 0.62; and roots 0.23 gm.

† Significantly different from controls.

The total increase in solid matter per plant during the entire experiment was not appreciably affected by treatment with the acid. During the first 5 days following treatment there was, in fact, a slight decrease in total solid matter. Table 3 shows that plants which were treated with 0.002 per cent acid lost 6.5 per cent of their original weight; those treated with 0.01 per cent solution, 5.5 per cent

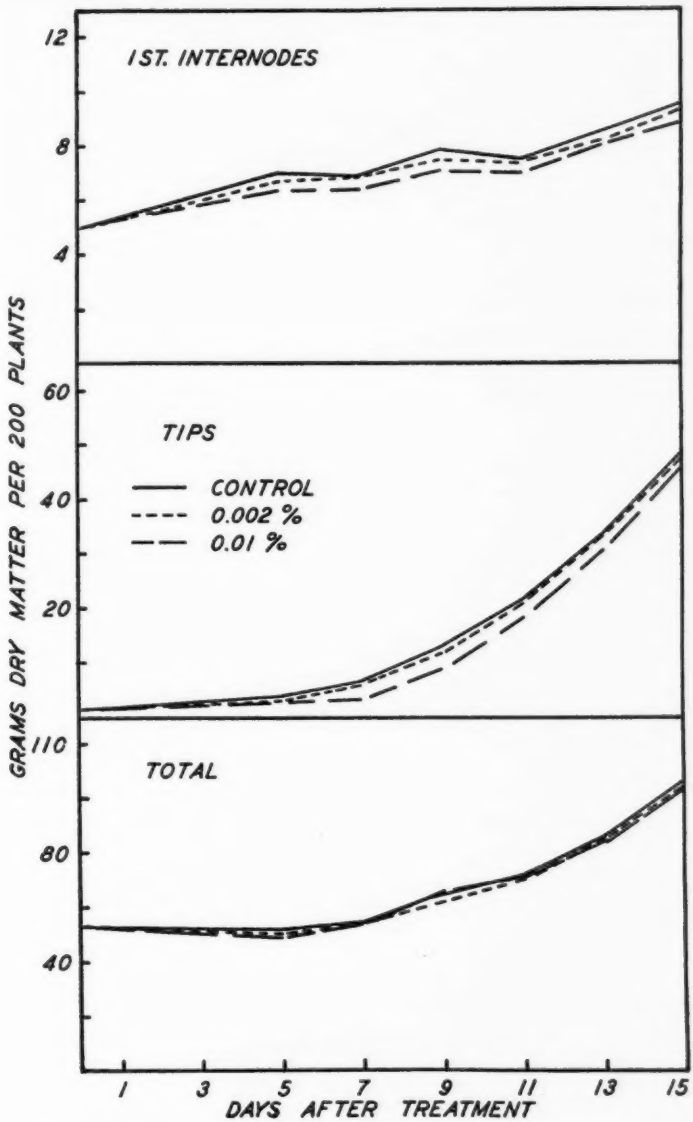

FIG. 3.—Dry weights of various parts of cuttings treated with indoleacetic acid solutions as compared with those of untreated plants.

of their initial weight; while untreated plants decreased only 3.2 per cent in dry weight during the same period of time.

There was at first a very apparent difference in the size of treated as contrasted with untreated cuttings, but this difference in appearance gradually became less and was hardly noticeable at the end of the experiment. As contrasted with this, approximately the same total amount of solid matter was synthesized by both treated and untreated plants. Thus the height and possibly the leaf area of control plants increased during the early part of the experiment more rapidly than did treated plants, but the total amount of solid matter synthesized was unaffected by treatment.

The percentage of moisture in treated cuttings was approximately the same as that of control plants between the fifth and fifteenth day after treatment, and the fresh weights of the total or of the different parts of treated and untreated cuttings show essentially the same results as have been described in connection with dry weight measurements.

In general the growth and appearance of cuttings treated with 0.002 per cent acid was not appreciably different from that of control plants at the end of a 15 day growing period following treatment. As contrasted with this behavior, the growth of cuttings treated with 0.01 per cent acid was accelerated in the region of root initiation, during the 5–7 days following treatment; the growth of the stems, petioles, and leaves was retarded for a longer period of time following planting than was that of untreated plants.

During the 5 days immediately following treatment, the total dry weight of stem tips, leaves, petioles, and first internodes of control plants decreased 6.3 per cent while that of the hypocotyls increased 11.4 per cent of their original weight. As compared with these figures, similar parts of cuttings treated with 0.01 per cent acid decreased 13.8 per cent and gained 36.4 per cent, respectively. The stimulating effect of indoleacetic acid on cell division and root initiation in the hypocotyl and the downward transport of food materials was only temporary, and these responses did not appreciably affect the synthesis of solid matter by the entire plant.

INFLUENCE OF INDOLEACETIC ACID ON METABOLISM OF CARBOHYDRATES.—It can be seen from figure 4 that the process of root for-

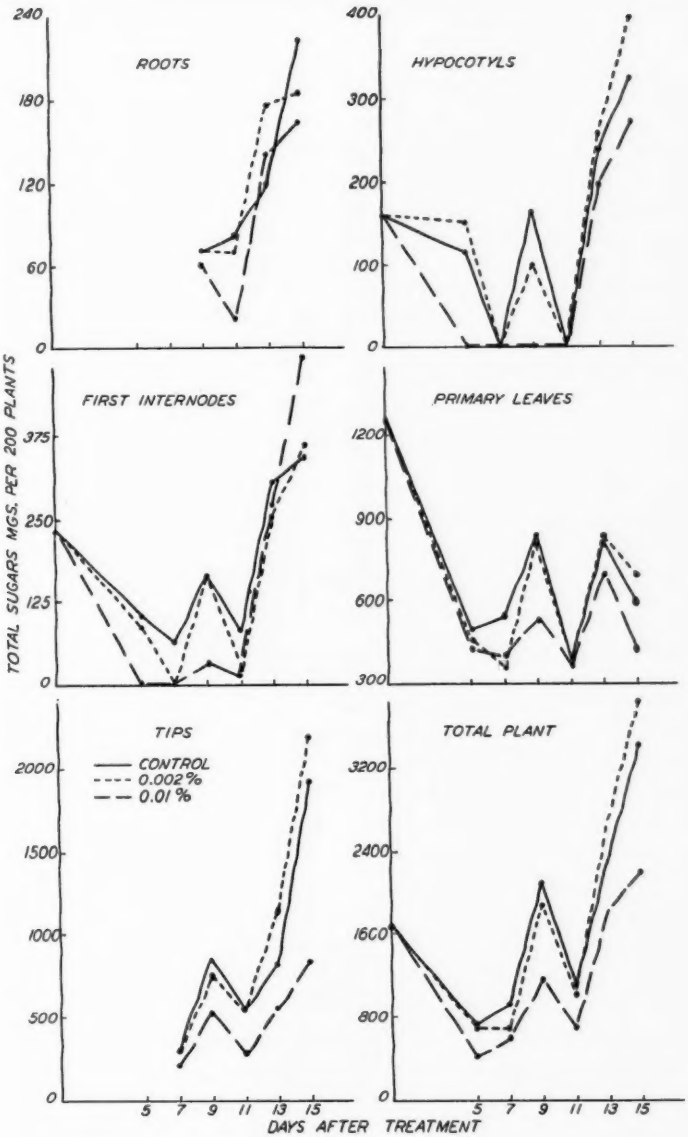

FIG. 4.—Effect of indoleacetic acid on sugar content of various portions of bean cuttings as compared with that of untreated cuttings.

mation on untreated cuttings was accompanied by a reduction in the sugar content of the cutting as a whole. This reduction was more pronounced in cuttings treated with indoleacetic acid, presumably owing to increased meristematic activity and root formation.

After root emergence on the fifth day following treatment, the leaf area increased rapidly and sugars accumulated in all of the cuttings for the duration of the experiment. The process of sugar accumulation was continuous with but one exception. Between the ninth and eleventh days a sharp reduction in sugar content took place in all the cuttings. The uniformity of this response suggested a variation in some environmental factor. While no significant difference in greenhouse temperature occurred at this time, there was a great reduction in light intensity on the ninth and tenth days after treatment. This reduction, as measured by a recording pyroheliometer, amounted to from 70 to 80 per cent of the light intensity on other days of the experiment, and is doubtless responsible for the lower sugar content of the cuttings harvested 11 days after treatment.

Table 3 shows that at the end of the 15 day growth period the treated cuttings weighed only slightly less than the controls. There were, however, large differences in the sugar content. On the thirteenth and fifteenth days the sugar content of cuttings treated with a 0.002 per cent acid was greater than that of untreated plants (figs. 4, 5). Prior to this time control plants contained slightly greater amounts of sugar. It is possible that the treatment with weak indoleacetic acid actually increased the rate of photosynthesis during the latter portion of the experiment. MITCHELL and HAMNER (7) noted that under certain conditions very low concentrations of indoleacetic acid-lanolin paste applied to the second internodes of intact bean plants stimulated synthesis of dry substance in excess of that produced by untreated plants.

Cuttings treated with 0.01 per cent acid contained less sugar than the controls throughout the course of the experiment. This lower content was not due to sugars being condensed to starch, as tests were made on all samples and starch could not be detected in the leaves, stem tips, or roots, and only traces were found in the

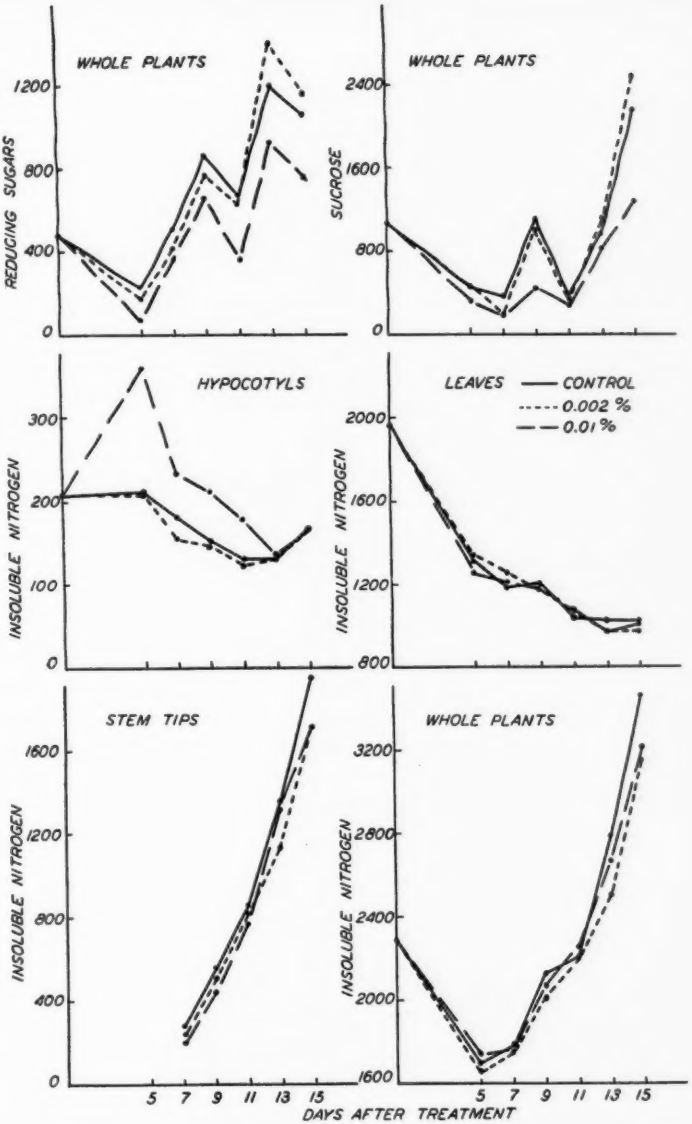

FIG. 5.—Effect of indoleacetic acid on sugar and insoluble nitrogen content of various portions of bean cuttings as compared with that of untreated cuttings. Plotted on basis of milligrams per 200 plants.

hypocotyls and first internodes. It is possible, however, that the sugars may have been condensed into polysaccharides other than starch, particularly in tissues near the point of treatment. Thus at the time of final harvest the dry weight of the roots of 200 control plants was 6.66 gm. as compared with 7.12 gm. for plants treated with 0.01 per cent acid. The amount of total sugars, on the other hand, was 222 mg. for controls and only 162 mg. for 200 treated plants. At the final harvest the dry weights and sugar content of the hypocotyls showed a similar inverse relationship. This difference between dry weight and amount of sugar could not be accounted for by nitrogen, as table 4 shows the nitrogen content of roots and hypocotyls of treated and untreated plants to be essentially the same at the time of final harvest. It is also of interest in this connection that the dry weight of the hypocotyls of plants treated with 0.01 per cent acid increased 36 per cent during the 5 days following treatment, while their sugar content decreased to approximately zero during the same interval. Although no direct measurements have been made, it is probable that the activity of enzymes concerned in the formation of certain polysaccharides, such as those present in cell walls, was accelerated through the use of indoleacetic acid. ALEXANDER (1) has reported that bean plants treated with indoleacetic acid-lanolin contained larger amounts of acid hydrolyzable polysaccharides, particularly near the region of application.

The initial cuttings contained more than twice as much sucrose as reducing sugars (table 4, fig. 5). Frequently throughout the course of the experiment sucrose was the only sugar present in certain portions of the cuttings. Thus there was no detectable amount of reducing sugars present in hypocotyls of either treated or untreated plants until the thirteenth day after treatment. Likewise, although the first internodes of the initial cuttings contained reducing sugars, none were found subsequently until the thirteenth day. Reducing sugars were always present in the leaves and predominated in the stem tips until the fifteenth day, when large quantities of sucrose were stored in the tips. The stem tips of both treated and untreated plants contained a much greater amount of sucrose and reducing sugars than did the roots of these plants.

In general the results show that when the plants were considered

as a whole the sugars decreased in all of the cuttings during the 5 day period immediately following treatment, the period when roots emerged. After the roots had emerged there was an apparent increase in the rate of leaf expansion, and sugars increased in all of the cuttings but were at all times lower in those receiving the strong treatment than in the controls (fig. 4). There is also some evidence that the weak treatment increased the sugar content, expressed either as percentage or absolute amount, over that in the control plants (figs. 4 and 5, table 4).

INFLUENCE OF INDOLEACETIC ACID ON METABOLISM OF NITROGEN. —The cuttings were not supplied nutrient solution containing nitrogen until after they had rooted; that is, on the sixth day. During this period of root initiation and emergence, nitrogen was mobilized from the leaves to the hypocotyls of both treated and control plants, but the rate of transport was stimulated as a result of the indoleacetic acid treatment, the stronger treatment being the more effective (fig. 6). Thus the hypocotyls of plants treated with 0.002 per cent acid gained twice as much and those treated with 0.01 per cent acid gained six times as much total nitrogen as did those of untreated plants during a period of 5 days following treatment. A negligible amount of this nitrogen that accumulated in the hypocotyls of control plants, and also those treated with 0.002 per cent acid, was present as insoluble nitrogen. In contrast to this, 66 per cent of the nitrogen accumulated, during the 5 days following treatment, in the hypocotyls of plants treated with 0.01 per cent acid was converted into an insoluble form.

After the initiation and emergence of roots, nitrogen steadily decreased in all of the hypocotyls, reaching a point where they contained less than at the beginning. The decrease was most rapid in the hypocotyls given the strong treatment in which all of the mobilized nitrogen disappeared. The implication is that the treatment with indoleacetic acid increased the proteolytic enzyme activity.

The reduction in nitrogen content of the hypocotyls of cuttings that received the strong treatment was equivalent to the amount deposited in the roots produced by these hypocotyls. In the case of those given the weak treatment, however, additional nitrogen besides that withdrawn from the hypocotyls was found in the roots.

TABLE 4

COMPOSITION OF KIDNEY BEAN CUTTINGS TREATED OCTOBER 14 WITH WATER, 0.002 PER CENT (WEAK), AND 0.01 PER CENT (STRONG) INDOLEACETIC ACID FOR THREE HOURS

Days after treatment	Roots			Hypocotyls			First internodes			Leaves			Tips			Total		
	Control	Weak	Strong	Control	Weak	Strong	Control	Weak	Strong	Control	Weak	Strong	Control	Weak	Strong	Control	Weak	Strong
	Total nitrogen (mg.) per 200 cuttings																	
0	.....	.....	.....	597	.....	.....	309	.....	.....	2805	.....	.....	104	.....	.....	3815	.....	.....
5	.....	.....	.....	634	671	828	456	453	469	2321	2210	2156	239	207	155	3650	3541	3608
7	73	78	166	591	624	748	450	476	431	2124	2237	2134	406	387	297	3644	3802	3776
9	130	128	203	535	574	612	446	457	453	2034	1951	1984	782	727	630	3927	3837	3882
11	164	160	272	468	478	572	384	381	382	1753	1766	1865	1308	1295	1217	4077	4080	4308
13	239	248	284	437	461	510	349	346	379	1640	1718	1766	1855	1837	1789	4520	4610	4728
15	322	306	336	424	438	488	317	331	324	1544	1554	1601	2530	2516	2491	5137	5145	5240
	Insoluble nitrogen (mg.) per 200 cuttings																	
0	.....	.....	.....	208	.....	.....	102	.....	.....	1970	.....	.....	.....	.....	.....	2280	.....	.....
5	.....	.....	.....	213	209	362	173	116	120	1310	1332	1259	.....	.....	.....	1696	1657	1741
7	.....	.....	.....	181	157	233	140	87	115	1189	1256	1215	274	251	202	1784	1751	1765
9	80	83	119	153	147	213	124	98	110	1209	1170	1177	564	516	452	2130	2014	2071
11	100	102	168	131	124	178	86	83	82	1036	1061	1064	857	826	770	2210	2196	2262
13	158	149	147	132	131	134	98	104	101	1030	984	975	1368	1139	1319	2786	2507	2676
15	204	207	209	172	169	170	110	93	106	1027	973	1010	1954	1709	1720	3467	3151	3215

DIRECT REDUCING SUGARS (MG.) PER 200 CUTTINGS

0				None			60.0			425						485		
5				None	None	None	None	None	None	229	177	72.5				229	177	72.5
7				None	None	None	None	None	None	266	181	206	246	233	168	512	414	374
9	19.2	28.9	29.2	None	None	None	None	None	None	295	305	259	549	440	370	863	774	658
11	56.4	48.7	Trace	None	None	None	None	None	None	230	190	167	387	390	196	673	629	363
13	72.8	95.9	100	104	90.2	33.1	127	92.3	69.9	411	484	394	487	655	331	1202	1417	928
15	148	122	120	125	150	128	178	174	93	264	367	176	346	354	251	1061	1167	768

SUCROSE (MG.) PER 200 CUTTINGS

0				151			161			774						1086		
5				110	145	Trace	101	85.5	Trace	235	250	326				446	481	326
7				None	None	None	60.9	None	None	241	153	169	63.1	59.8	19.8	365	213	189
9	46.8	38.7	27.5	155	97.2	None	158	154	34.2	407	457	246	278	274	138	1135	1021	446
11	20.4	17.0	20.5	Trace	None	None	75.2	22.1	17.7	142	167	164	141	126	81.5	379	332	284
13	37.3	70.2	31.8	123	154	153	161	147	165	358	307	274	300	435	196	979	1113	820
15	63.9	52.0	34.2	184	226	131	150	170	373	294	289	212	1469	1713	538	2161	2450	1288

TOTAL SUGARS (MG.) PER 200 CUTTINGS

0				159			235			1272						1666		
5				116	152	Trace	106	89.5	Trace	496	457	426				718	699	426
7				None	None	None	63.7	None	None	545	362	403	324	307	198	933	669	601
9	70.9	72.3	60.7	163	102	None	167	162	36.3	834	806	539	861	746	530	2096	1888	1166
11	81.8	70.0	21.5	Trace	None	None	79.7	23.6	18.4	303	380	359	555	541	291	1110	1015	690
13	118	176	140	240	257	197	304	253	249	808	830	704	827	1139	558	2297	2655	1848
15	222	184	162	325	395	273	344	363	492	589	696	418	1920	2181	842	3400	3819	2187

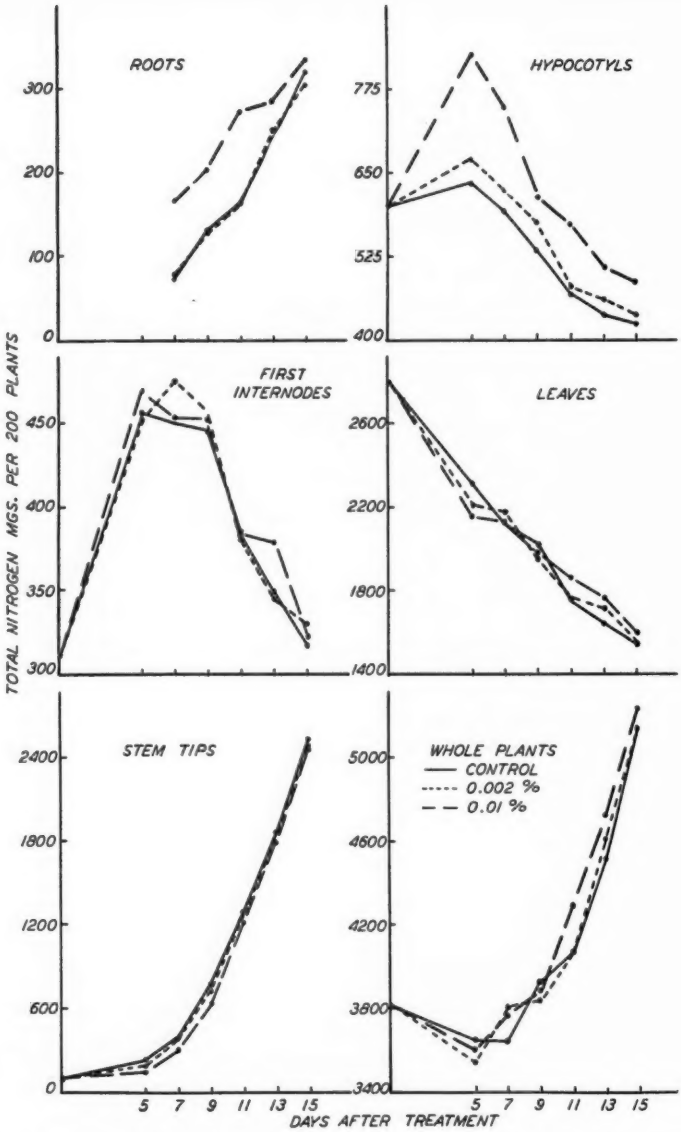

FIG. 6.—Effect of indoleacetic acid on total nitrogen content of various portions of bean cuttings as compared with that of untreated cuttings.

Since nitrogen was supplied in the nutrient solution after the roots had emerged, the exact source of the nitrogen in the roots is uncertain.

Nitrogen from the leaves accumulated also in the first internodes of all the cuttings during the initiation and emergence of roots. In contrast to the nitrogen content of the hypocotyls, the accumulation was nearly as great in the controls as in the treated cuttings. After the roots had emerged, nitrogen was translocated from the first internodes to other parts of the cuttings (fig. 5).

It is of interest that during the period of root initiation some of the nitrogen which was translocated from the leaves moved to the hypocotyls and first internodes. After root emergence, however, the nitrogen which continued to move from the leaves apparently entered the growing stem tips (fig. 5). The withdrawal of nitrogen from the leaves was continuous throughout the experiment, even after additional nitrogen from the nutrient solution was supplied through the roots. However, figure 5 shows that there was very little decrease of insoluble nitrogen (protein hydrolysis) in the leaves after the eleventh day. By this time the roots had developed sufficiently to absorb appreciable amounts of nitrogen from the nutrient solution.

The nitrogen content of the tips increased from 3 per cent of the total nitrogen in the initial cuttings to almost 50 per cent of the nitrogen in the plants at the end of the experiment. Treatment with indoleacetic acid resulted in slightly less growth of the tips than in the controls. As a result, the total amount of nitrogen in the tips from the treated cuttings was always less than in the control tips. This difference decreased after the ninth day and at the end of the experiment was less than 2 per cent.

Applications of nitrogen as a nutrient increased the nitrogen content of the cuttings by about 35 per cent. The amount of nitrogen absorbed by cuttings treated with 0.01 per cent acid was not appreciably greater than that absorbed by control cuttings, although approximately 60 per cent more roots emerged from hypocotyls of the former. Ash determination made on the tips and leaves of the plants at the end of the experiment showed that 200 treated plants contained but 520 mg. more ash than similar portions of control

plants. The increased root production did not appreciably influence the uptake of nutrients. It should be pointed out that although plants treated with the strong acid produced a greater number of roots than did the controls, the dry weight of roots of cuttings that received the stronger treatment was only slightly greater than that of the controls at the end of the experiment.

The slight difference in weight in favor of the control plants at the end of the experiment is reflected chiefly in the tips. This result is attributable, in part at least, to an influence of the acid on the carbohydrate metabolism of the cuttings. In so far as the nitrogen metabolism is concerned, the indoleacetic acid effect is most noticeable during the process of root initiation and emergence.

Discussion

It is known that some internal factors can modify the response of plants to indoleacetic acid. Thus the duration of increased protoplasmic streaming induced in the cells of some plants by low concentrations of auxin is affected by the amount of available sugar (12). The growth of oat coleoptiles, induced by the application of auxin, is also dependent upon available sugars and possibly upon the presence of certain salts (13). It is probably true that many other organic and inorganic substances generally classed as nutrients might also modify the response of certain plants to auxin treatment, although there is at present little proof of this assumption. Experiments on the rooting of cuttings (3, 14) have shown that indoleacetic acid possibly acts as a mobilizer of naturally occurring root forming substances, and that the lack of these substances could also limit the rooting response of a cutting to indoleacetic acid treatment.

Recent investigations have shown that if all of the necessary factors are present, and indoleacetic acid is applied in appropriate concentrations to some plants in the form of a paste, then many of the cells near the point of treatment increase in size, their protoplasm becomes more dense, and cell division occurs (5). If this chain of reactions which leads to the formation of new cells is initiated in one portion of the plant, by the use of indoleacetic acid, then the necessity for an adequate supply of foods in that part becomes, temporarily at least, very great. When the acid is applied to cuttings which

root readily without treatment, such as those of kidney beans, then the processes of cell division and root formation are stimulated above that of untreated cuttings, and there is necessarily a need for additional nutritive substances out of which new cells can be formed in the treated portion. Thus in the experiments reported here, carbohydrate and nitrogenous compounds moved from even remote parts of the plants to the point where cellular division was stimulated. This movement of compounds from one part of the plant to be combined into new cells formed in another part becomes an important factor, particularly in connection with the rooting of cuttings, as it concerns the general nutrition and growth of the plant.

It is therefore of considerable practical importance to determine whether the propagation of cuttings with growth substances affects their subsequent growth and behavior after rooting. An earlier paper (11) discussed the effect of treatment with indoleacetic acid on the nitrogen and carbohydrate metabolism of kidney bean cuttings during the rooting process. The present experiment was planned to secure information concerning the effect of the acid on the growth and metabolism of bean cuttings after they were rooted.

In order to evaluate the effect of the treatment on growth of the cuttings, it was necessary to set up the experiment in such a manner as to permit statistical interpretation of the growth data. These data show that neither of the concentrations here used increased the growth of the cuttings in comparison with the controls. It is possible, of course, that cuttings of other species might react differently. As previously pointed out, there was an initial stimulation in growth of the roots and hypocotyls as a result of treatment, accompanied by less growth of the stem tips and first internodes on the treated cuttings during the rooting process. It was during this period that striking differences in distribution of nitrogen and carbohydrates were noted. The difference between the weak and strong treatment, as well as results previously obtained (11), indicates that this initial growth response is dependent upon the strength of the indoleacetic acid as well as the length of exposure of the cuttings to it.

The initial influence of the acid was modified during the later growth of the plants. Thus at the end of the experiment there was an insignificant difference between the weights of the various parts

of the treated cuttings and the corresponding parts of the controls, with the exception of the stem tips and first internodes. This difference in growth was not due to a deficiency of nitrogen, since the treated cuttings contained slightly more nitrogen than the controls. As far as the carbohydrates are concerned, the weak treatment with indoleacetic acid resulted in an apparent increase of sugar content, while the strong treatment significantly reduced the sugars in the cuttings in comparison with the controls. It is not possible to interpret these findings with respect to the carbohydrate metabolism fully until the relative influences of the acid on the rate of photosynthesis, respiration, and enzymatic activity are better understood.

The present investigation shows that treatment of kidney bean cuttings with indoleacetic acid results in a prompt and distinctive growth response until the cuttings are rooted. After that period, the stimulating and modifying effects of the acid on growth tend to diminish during the subsequent vegetative development of the plants until little difference exists between the treated and control plants at the end of the experiment.

Summary

1. Cuttings of kidney bean seedlings were treated by immersing their bases in water, 0.002 per cent, or 0.01 per cent indoleacetic acid for 3 hours. They were then set in quartz sand contained in 4 inch clay pots. The pots were systematically distributed on each of two greenhouse benches which were then inclosed by glass to maintain a relatively high humidity. Cuttings were harvested 5 days after treatment and every second day thereafter until 15 days had elapsed. Each sample was divided into roots, hypocotyls, first internodes, primary leaves and petioles, and tips. Fresh weight, dry weight, total and insoluble nitrogen, reducing sugars, and sucrose were determined for each sample.

2. Treatment with 0.01 per cent indoleacetic acid significantly increased the weight of the hypocotyls and roots over the corresponding portions of the controls until the thirteenth day after treatment. The same treatment significantly decreased the growth of the first internode throughout the course of the experiment and also the

growth of the tips, and the difference became statistically significant on the ninth day and remained approximately constant through the rest of the experiment. Treatment with even a strong solution (0.01 per cent) of acid did not appreciably affect the dry weight of the primary leaves. Weak treatment (0.002 per cent) caused slight growth responses that were qualitatively like the strong treatment but statistically insignificantly different from the controls. Neither the weak nor the strong treatment increased the weight of the whole plant above that of the control.

3. During the rooting process nitrogen was transported from the leaves to the first internodes and hypocotyls, the strong treatment being by far the most effective in increasing the amount mobilized. This nitrogen was subsequently translocated to other portions of the cuttings. In the case of cuttings treated with 0.01 per cent acid, a large amount of this mobilized nitrogen was temporarily deposited in the hypocotyls in an insoluble form, but it was subsequently translocated to other portions of the cuttings. Since all of this accumulated nitrogen subsequently disappeared, it is evident that the treatment stimulated the proteolytic enzymatic activity of the cuttings.

4. Application of nutrient solution after the cuttings were rooted increased their nitrogen content by about 35 per cent. The larger number of roots induced as a result of treatment resulted in the uptake of only a slightly greater amount of nitrogen and inorganic substances than were absorbed by the controls.

5. The treatment of cuttings with 0.002 per cent acid resulted in slightly greater accumulation of sugars than in the controls at the end of the experiment. Treatment with 0.01 per cent acid significantly reduced the sugar content of cuttings below that of controls at all times. No starch was present at any time in the roots, leaves, or tips, and only traces in the hypocotyls and first internodes. Interpretation of the carbohydrate metabolism in relation to growth requires further information as to the effect of the acid on the rates of photosynthesis, respiration, and enzymatic activity.

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EFFECT OF PHOTOPERIOD ON DEVELOPMENT AND METABOLISM OF THE BILOXI SOY BEAN

M. W. PARKER[1] AND H. A. BORTHWICK[2]

(WITH SEVENTEEN FIGURES)

Introduction

Several chemical studies have been reported of soy beans that have been subjected to different photoperiods. In most of these the object has been to explain, on a chemical basis, the cause of the visible changes that are induced by variations in photoperiod. In some of these experiments attention has been given to the morphological condition of the apical meristems of the plants before and during the experiments, but in others such observations have been lacking. In some cases plants supposedly vegetative were probably developing flowers. In others it is apparent that the samples were taken well after flower bud differentiation was completed. In such cases chemical differences between the plants on long and short photoperiod would not necessarily indicate the cause of the initial photoperiodic response. Thus far the results of such experiments have not furnished an explanation of the photoperiodic reaction, nor have they indicated that such an explanation would ultimately come from this particular type of experiment. It has seemed desirable, nevertheless, to repeat certain experiments with slight modification and give special attention to stages of floral development in the experimental material at the times of sampling.

In making a detailed chemical study of the metabolism of Biloxi soy beans subjected to differential photoperiodic treatments, it was necessary to make a choice between a large number of treatments sampled relatively infrequently and a smaller number sampled more often. To obtain critical data, the study was restricted to four types of treatment each of which would produce a certain morphological response. In one, early flowering and fruiting was desired. In

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another the plants were to be kept in a vegetative condition. Information already available indicated that 8 hour photoperiods for the former and 16 hour photoperiods for the latter were suitable. In the other two lots, on which flower primordia were to be initiated, one was to be placed on photoperiods above the critical and the other on photoperiods below the critical. In these two lots maximum flowering and fruiting was desired on one and complete suppression of flowering and fruiting on the other. The purpose of the experiment in part I was to determine the photoperiods necessary to produce these results.

The effect of photoperiod on the carbohydrate and nitrogen metabolism on various plants has been determined by several investigators (**1, 2, 6, 9, 14**). In some of these investigations the plants used had already flowered and in some cases fruited. Although differences in composition have been reported in plants that have received different photoperiodic treatments, these differences in general appear to be associated with, rather than a cause of, the morphological response induced by photoperiod. Changes in chemical composition that can stand in a causal relationship to initiation of flower primordia must occur before initiation takes place. In experiments where the samples were taken at the time of flowering, reactions of a causal nature may have been obscured by changes in metabolism that occurred subsequent to initiation. MURNEEK (**8**), working with the Biloxi soy bean, obtained samples for chemical analysis both before and after initiation of flower primordia. He reports that changes in the relative amounts of carbohydrate and nitrogen compounds do not appear to be of sufficient magnitude to account for the initiation of flower primordia. For satisfactory fruiting, however, he suggests that a favorable relationship between carbohydrate and nitrogen compounds may be essential.

MURNEEK also subjected Biloxi soy beans to so-called "switch-over" experiments, in which he transferred plants that had been growing on either a long or a short photoperiod to one of the opposite length. When very young plants were used for such experiments the metabolism of plants transferred to a given photoperiod from another soon became typical of that of plants which had been grown continuously on that photoperiod.

Since the development of soy beans after the initiation of flower primordia is dependent upon the photoperiod to which they are subjected at that time, a study of the metabolism of plants transferred to various photoperiods after initiation might explain the different growth responses. In the investigation here reported a group of plants that had been grown on long photoperiods and were consequently vegetative, were given a week of short photoperiods to initiate flower primordia. They were then transferred to various photoperiods, the lengths of which were selected to give differences in rate of development of flowers and fruits. The carbohydrate and nitrogen metabolism and the morphological development of these plants have been determined.

I. Effect of various photoperiods on development following initiation of flower primordia

Material and methods

In this experiment Biloxi soy beans were grown on 16 hour daily photoperiods for 4 weeks, conditions under which these plants remain vegetative. At the end of this time all plants except a few controls kept on the 16 hour photoperiod were subjected to seven photoperiods of 8 hours each. In response to this treatment flower primordia were initiated. These plants were then divided into eight groups and subjected to various photoperiods ranging in length from 8 to 18 hours for the remainder of the experiment. This range gave several lots below the critical day length of approximately 14 hours and several above.

The photoperiods of all plants were started at 8 A.M. each day. At the end of the photoperiod the plants, which were kept on warehouse trucks, were moved into a darkhouse constructed within the greenhouse. In all except the 8 and 10 hour lots the photoperiod was extended with Mazda light of approximately 40 foot candles.

Experimental results

During the course of the experiment weekly measurements of heights of representative plants from the different photoperiods were recorded, and frequent observations of the stage of development of flower primordia on plants of the various lots were also

made. At the conclusion of the experiment certain yield data were obtained.

The first height measurements were made at the end of the induction period. In making these records plants were selected from each lot and measured throughout the experiment. On June 2, when the first measurements were taken, the leaf at node 8 was in the process of expansion. The distances from the cotyledonary scars to the base of this leaf and to the tip of the terminal bud were both

TABLE 1

WEEKLY INCREASE IN LENGTH OF STEM FROM COTYLEDONS TO EIGHTH NODE

PHOTOPERIODIC TREATMENT	WEEKLY INCREASE IN LENGTH PER PLANT		
	JUNE 2–8	JUNE 8–15	JUNE 15–22
Controls			
16 hour	6.4	1.4	0.0
Transfers			
8 hour	5.6	2.6	0.1
10 hour	5.8	1.8	0.0
12 hour	7.0	3.2	0.0
13 hour	8.8	3.3	0.0
14 hour	8.3	1.9	0.0
15 hour	8.6	1.4	0.0
16 hour	9.3	1.1	0.1
18 hour	9.2	2.4	0.3

recorded. This gave separate records for the portion of stem in which elongation was nearing completion and the part in which most of the remaining growth was still to occur. The results are presented in the form of weekly gains per plant in centimeters (tables 1 and 2).

Elongation of the part of the stem below the eighth node was completed in most cases by June 15, the end of the second week after the induction treatment was finished. During the first week after induction considerable elongation was still occurring in this region, and the amount seems to be correlated with the length of photoperiod received during that week.

The upper portion of stem was so short on June 2 that during the following week it made relatively little elongation. Even so there was still a direct correlation between the gain made and the length of

photoperiod. During the next 3 weeks the gains were very much greater and the relationship previously observed between amount of gain and length of photoperiod still prevailed. This effect was most pronounced at photoperiods of 8 to 13 hours duration, inclusive. Above that point the differences were variable and not great. During the fifth week after induction, growth in length of the 8, 10, and 12 hour plants virtually ceased and was greatly diminished in the 13 hour transfers. Growth in the lots on photo-

TABLE 2

WEEKLY INCREASE IN LENGTH OF STEM FROM EIGHTH NODE TO TERMINAL

Photoperiodic treatment	Weekly increase in length per plant				
	June 2–8	June 8–15	June 15–22	June 22–29	June 29–July 6
Controls					
16 hour....	2.5	13.9	14.2	10.8	14.2
Transfers					
8 hour....	0.2	6.2	6.5	8.5	1.6
10 hour....	1.1	9.0	9.8	6.0	0.8
12 hour....	1.5	11.4	13.5	10.9	2.0
13 hour....	1.9	10.3	19.2	14.7	5.6
14 hour....	2.5	13.5	18.8	17.5	14.2
15 hour....	2.5	15.8	19.4	13.5	9.8
16 hour....	3.1	13.9	19.4	16.3	8.8
18 hour....	2.6	16.1	18.3	15.0	9.2

periods of 14 to 18 hours, however, continued at a rate only slightly lower than during the previous weeks.

After the plants had been grown 4 weeks on 16 hour photoperiod, microscopic examinations of the terminal meristems of representative plants were made and no flower primordia were present. The plants were then subjected to an induction period consisting of 8 hour daily photoperiods for 1 week, at the end of which time twenty more plants were examined for flower primordia.

The plants at this stage of development had an average of 17.3 nodes per plant in the main axis and flower primordia were abundant. These structures did not yet have the initials of the various cycles of flower parts, but the primordia of the bract at the base of each flower and of the two bracts situated laterally on each flower

just below the calyx were evident. Between and above these latter bracts was a mass of meristematic tissue which in a few days more differentiated into the receptacle of the flower with its various cycles of floral organs.

The flower primordia were usually found in the axil of the fifth leaf from the apex of the main stem and in similar positions in several axillary buds. In most plants they were present in the axil of the thirteenth node from the base of the main axis; in others they were in the axil of the twelfth. They were also found in buds located at nodes 3 to 9 inclusive, being present most frequently in the buds at nodes 6, 7, and 8.

No flower primordia were found at this time in the buds located at nodes 10 and 11. This condition, in which flower primordia are absent from buds at certain nodes but present both above and below, is a regular occurrence in the soy bean if examination of the plants is made within a week after the start of treatment with short photoperiods. In the present case the buds at nodes 10 and 11 had begun to differentiate primordia of vegetative leaves at the time treatment was started. Most frequently there were two vegetative leaf primordia in the bud at node 11, and three at node 10. Differentiation of these structures into vegetative leaves was evidently sufficiently far advanced a week previously, when 8 hour photoperiods were first applied, so that their development into floral bracts was impossible. The buds in the axils of these leaf primordia, on the other hand, had not yet developed far enough to determine whether they were destined to be vegetative or floral in character.

On June 8 dissections were made of plants on each photoperiod. Many more flower primordia were visible at this time on all lots, but there were no obvious differences in the total number present at any of the photoperiods. Comparison of the stages of development of flower buds at comparable positions on plants of the various lots showed little difference between them. All the floral organs were present in the first formed flower buds on the plants of each lot. The individual structures, however, were not far advanced. The pistil was represented by an infolded ridge in which differentiation into stigma, style, and ovary was not yet apparent. Sepals and petals were well developed, but differentiation of the anthers was just beginning.

On June 15 the flower buds in all lots were much further developed but had not yet opened. Measurements were made of the length of buds taken from identical positions on plants of each photoperiod. It was found that the buds from plants grown on photoperiods of 13 hours or less were approximately twice as long as those grown on the longer photoperiods.

Aceto-carmine smears of the anthers of many buds from plants of each group showed microspores or microspore mother cells in stages of division in the largest buds of all plants on photoperiods of 13 hours or less. In the plants on longer photoperiods mother cells were not yet fully differentiated except in the case of one plant on 14 hour photoperiod, and in this one reduction divisions had not yet begun.

On June 22 flowers were open on some of the 13 hour plants. The flowers were normal in size and appearance in contrast to those on plants grown on shorter photoperiods. In the latter the corollas failed to open in the customary manner but simply elongated slightly beyond the tips of the calyx and remained unopened. A few days later the expanding pods emerged beyond them.

Smears were again made of anthers from the largest unopened buds of each lot. Pollen grains were found in all lots from the 8 to 14 hour ones, inclusive. The plants on longer photoperiods were somewhat variable in their stage of development, but most of them either had no visible mother cells or they were just differentiating. The largest buds from plants on photoperiods of 8 to 13 hours inclusive were about 4 mm. long and those from longer photoperiods were 2 to 3 mm. long.

On June 29 all plants on photoperiods of 13 hours or less, and forty-two out of fifty-two on the 14 hour photoperiod, were in bloom. Observations on anthers of plants on the longer photoperiods showed that pollen grains had finally formed in the largest buds of all of them. The buds were still only about 3.5 mm. long, however.

Pods 2 to 3 cm. long were present on plants from the 8, 10, and 12 hour photoperiods. On the 13 hour photoperiods pods were just beginning to appear.

On July 6 all of the 14 hour plants and occasional plants from the 15 hour lot were in bloom. Flowers were not abundant on the plants of either lot, however, and no pods were developing.

On July 14 the plants of all lots were harvested. Fresh weights of the leaves, stems, and pods, and records of the number of pods per plant were made. These results appear in tables 3 and 4. There was an increase in fresh weight per plant as the photoperiod was increased from 8 to 13 hours per day. Beyond this point no signifi-

TABLE 3

FRESH WEIGHT OF PLANTS HARVESTED JULY 14

Photopericdic treatment	Total plants	Mean fresh weight per plant in grams			
		Whole plant	Leaves	Stems	Pods
Transfers					
8 hour....	32	72.7	37.3	14.8	20.6
10 hour....	32	83.6	40.1	16.8	26.3
12 hour....	32	91.5	43.3	19.3	28.8
13 hour....	21	102.9	50.4	25.2	27.2
14 hour....	32	102.0	61.0	40.0	None
15 hour....	32	99.0	58.8	39.2	None
16 hour....	31	105.3	64.4	39.9	None
18 hour....	18	107.1	67.4	40.6	None

TABLE 4

POD YIELD ON VARIOUS PHOTOPERIODS

Photoperiodic treatment	Mean no. of pods per plant	Mean weight of pods per plant (gm.)	Mean weight of 100 pods (gm.)
Transfers			
8 hour.....	26	21	79
10 hour.....	30	26	89
12 hour.....	28	29	101
13 hour.....	32	27	86

cant increase in fresh weight of whole plants occurred. The leaf and stem fractions of these plants showed a similar relationship to photoperiod, except that they showed further increase on 14 hour photoperiod.

Pods were present only on the 8, 10, 12, and 13 hour lots. The plants on the 8 hour photoperiod produced the smallest number of pods, the smallest weight of pods per plant, and the smallest weight per 100 pods (table 4). The other three photoperiods were not

greatly different from one another in pod yield. The individual pods, however, were somewhat heavier on the 12 hour photoperiod.

Discussion

In previous work (3) it has been shown that flower primordia can be initiated by Biloxi soy beans under a wide range of photoperiods. The critical photoperiod above which they are not initiated is about 14 hours. In the present work the photoperiods that have previously been shown to be most favorable for initiation are likewise most favorable for subsequent growth and development of flower primordia that have already been initiated. The flower buds grew much faster on the plants receiving photoperiods of 8 to 13 hours than on those receiving longer photoperiods. Continuous but slow growth of the buds occurred on 14 hour photoperiod and all of the plants finally bloomed. On 15 hour photoperiods a similar effect was observed, but at the time the experiment was discontinued not all of the plants had bloomed.

Initiation of flower buds has previously been observed on 14 hour photoperiod in the Biloxi soy bean (3) but not at 15 hours, except in very old plants. The results seem to show that growth of flower primordia in this plant can proceed on photoperiods longer than will permit their initiation.

The longest photoperiod at which abundant development of fruits occurred was 13 hours. The plants at this photoperiod resembled the 8 hour ones in their fruiting habit, but in the total amount of growth as measured by height and fresh weight they were more like the plants grown on longer photoperiods. The shortest photoperiod at which no flowering occurred even though flower primordia were known to be present at the beginning of the experiment was 16 hours.

II. Physiological and chemical studies

Cultural methods

The plants used in this experiment were grown out of doors in boxes of soil approximately 10 × 10 inches × $3\frac{1}{2}$ feet. Abundant seeds were planted in each box so that when the plants were thinned to ten per box, 13 days after planting, a uniform stand was ob-

tained with little or no transplanting. In all, 272 boxes were planted. Of these, 192 were placed on trucks that could be moved into adjacent darkhouses at will and the remaining 80 were placed in a group on the ground near these cars. Mazda lights, controlled by electric time switches, were installed over the 80 boxes and also in the darkhouses. With this equipment it was possible to extend the photoperiod of natural light any desired amount. The lights were turned on shortly before sundown and off again about 2 hours later. The time the lights were turned off was changed as the season advanced, thereby maintaining a 16 hour daily photoperiod for the duration of the experiment.

The Mazda lights used were 100 watt each and were spaced sufficiently close so that 30–60 foot candles of light was supplied to the leaf surface. It has previously been shown that this intensity of light, used to extend a natural photoperiod beyond the critical length, is far in excess of that required to prevent flowering in the Biloxi soy bean (4).

The planting was made May 24 and all the plants were grown on 16 hour photoperiods until July 5. Beginning on this date, the 192 boxes of plants on trucks were given seven daily photoperiods of 8 hours each. The 80 boxes not on trucks were continued on 16 hour photoperiods. These plants served as long day controls, and from the time the seedlings emerged from the soil until the plants were finally harvested they never received less than a 16 hour daily photoperiod.

On July 12, after the plants on trucks had received seven daily photoperiods of 8 hours each, these plants were divided into three lots, each of which received a different photoperiod from that date until the end of the experiment. One lot was shifted to a 16 hour, another to a 13 hour, and the third was continued on an 8 hour photoperiod (table 5).

Chemical methods

Sampling.—Samples of 60 plants each were drawn for chemical analysis July 5, 12, 19, 26, August 2, 9, and September 14. On July 5 a single sample was taken at random from the entire population. Since all the plants had received identical photoperiodic

treatments up to this time, additional samples were not needed. On July 12 two samples were selected at random, one from the 16 hour control plants and one from the thirty-two carloads that had received a week of 8 hour photoperiods. On each of the remaining sampling dates four separate samples were drawn, one from each of the lots shown in table 5.

All samples were taken at the beginning of the photoperiod of the date indicated. The beginning of the photoperiod of the various lots occurred at different times of the morning. This was advantageous since the sampling of one lot could be completed before time to harvest the next. The plants were cut at the cotyledonary nodes in all cases, and immediately brought to the laboratory. The fresh weight of the stems and the leaves was determined. The leaves were then cut into small segments and thoroughly mixed. From this a representative aliquot was withdrawn and dried in a forced draft oven at 70° C. The remaining tissue was ground in a food chopper. This grinding reduced the tissue to a very fine pulp, from which samples were drawn for analysis. The stems were treated in exactly the same manner as the leaves.

TABLE 5

SCHEDULE OF PHOTOPERIOD

Designation of lots	Photoperiod received at various times		
	May 24–July 4	July 5–July 11	July 12 to end of experiment
Controls			
16 hour.....	16 hour	16	16
Transfers			
16 hour.....	"	8	16
13 hour.....	"	8	13
8 hour.....	"	8	8

Moisture.—Samples consisting of 4–8 gm. of ground tissue were dried to constant weight in a vacuum oven at 80° C. under 2 to 3 cm. pressure.

Sugars.—The sugars were determined by a method previously used (10). In brief the method is as follows: A sample of the ground

pulp was weighed into a counterpoised 200 ml. Kohlrausch sugar flask and covered immediately with sufficient boiling 95 per cent alcohol to give approximately 80 per cent alcohol covering the sample. The samples were brought to a boil on the steam bath soon after adding the alcohol and sufficient boiling water was added to give approximately 60 per cent alcohol. The extraction on the steam bath continued for 30 minutes. Preliminary tests with soy bean tissue showed that this time was sufficient. After the extraction, the flask was removed and made to volume with cold 95 per cent alcohol. The volume was again adjusted in the same manner just before making a determination. The reducing sugars were determined according to the Official Method (7), using the Munson and Walker gravimetric procedure. Total sugars were determined by the Official Method of acid hydrolysis. The hydrolysis was allowed to proceed overnight in a warm place. The amount of sucrose present was calculated as prescribed in the Official Method.

STARCH.—The starch was determined by a modification of a method previously reported (10). Samples of tissue that had been dried in a forced draft oven at 70° C. were ground with a Wiley mill to pass a 40 mesh sieve. Samples of this ground tissue, after redrying, were extracted free of sugars by means of a Soxhlet apparatus. The weight remaining after extraction was determined and the samples were reground to pass an 80 mesh sieve. Samples of this tissue were used to determine starch by a modification of the Official Method, in which an enzyme is used with subsequent acid hydrolysis. Fresh diluted saliva (1:10) was used as the enzyme for each digestion period, and enzymatic digestions were continued until microscopic examination showed no starch present. The samples were made to 250 ml. volume and filtered. Two hundred ml. of this filtrate was placed in a 250 ml. volumetric flask and treated with sufficient neutral lead acetate to precipitate interfering substances. The solution was made to volume and filtered. After deleading with anhydrous sodium oxalate, the samples were again filtered. A 200 ml. aliquot of this cleared solution was hydrolyzed 2.5 hours with 12.5 ml. of concentrated hydrochloric acid. Reducing sugars were determined by the Munson and Walker gravimetric procedure (7), and starch was calculated as described in the Official Method (7).

No attempt was made to determine dextrins, consequently any present were included with the starch.

TOTAL NITROGEN.—Samples used to determine moisture were transferred to Kjeldahl flasks and used for total nitrogen determinations. Since nitrates were present in all samples, the reduced iron method (11) was employed. Following the reductions, the Gunning method (7) was used to complete the digestion. Distillation was made into 0.1 N sulphuric acid and the excess acid was titrated with 0.1 N sodium hydroxide using the combination methylene blue-methyl red indicator.

EXTRACTION OF NITROGENOUS CONSTITUENTS.—A 50 gm. sample of the ground leaves or stems was transferred to a mortar and thoroughly ground with nitrogen-free quartz sand, with gradual addition of water to insure good grinding. After grinding for 10 minutes the suspension was decanted to a square of huck toweling suspended over a 2 liter beaker. After allowing to drain and then expressing by hand, the residue was returned to the mortar. The same extraction procedure was repeated two additional times. This resulted in complete removal of the non-protein nitrogenous constituents. The combined extracts were heated just to boiling, three drops of 10 per cent acetic acid and 7 ml. of dialized iron containing 5 per cent Fe_2O_3 were added. After boiling 2 minutes, the solution was filtered while hot and the residue in the Buchner funnel was thoroughly washed with hot water. The filtrate was cooled, made to 1000 ml. volume, and preserved with toluene. This extract contained only non-protein nitrogen. The procedure is essentially the same as that reported by STUART (5).

NON-PROTEIN NITROGEN.—Duplicate 100 ml. aliquots of the non-protein nitrogen extract were analyzed as described for Total Nitrogen.

ALPHA-AMINO-NITROGEN.—A 200 ml. aliquot of the non-protein nitrogen extract was treated as described by STUART (13) to remove interfering substances. After concentrating in vacuo to 50 ml., the amino-nitrogen was determined by the Van Slyke micro-apparatus.

AMMONIA NITROGEN.—The free ammonia was determined by the aeration method of SESSIONS and SHIVE (12), using duplicate 100 ml. aliquots.

NITRATE NITROGEN.—Nitrate nitrogen was determined on the ammonia-free samples by the method of SESSIONS and SHIVE.

SOLUBLE ORGANIC NITROGEN.—The difference between the soluble non-protein nitrogen and the sum of the ammonia and nitrate nitrogen was designated soluble organic nitrogen.

PROTEIN NITROGEN.—The difference between the total nitrogen and the soluble non-protein nitrogen was designated protein nitrogen.

EXPERIMENTAL RESULTS

On each sampling date, terminals of twenty-five plants from each sample were dissected. From these dissections, data were obtained as to the total number of nodes per plant and as to the kind of structures present at the growing tips. In those lots where flower buds were present, observations of their stage of development and measurements of their size were made. Height measurements from the cotyledonary scars to the terminals of twenty plants in each of the four lots were recorded at weekly intervals (fig. 1). The plants used for this purpose were numbered, so that measurements could be made each week from the same plants.

From these data it is seen that the 16 hour controls and the 16 hour transfers both continued to increase in length at a fairly constant rate throughout the season. The controls always maintained a position slightly in advance of the 16 hour transfers. The 13 and 8 hour transfers also increased in length at practically the same rate as the controls and 16 hour transfers until August 2. During the following week the rate decreased, however, and by August 16 elongation had ceased.

This behavior of growth in length is associated with the flowering and fruiting response made by the various lots. Although flower primordia were present on all of the plants in the three transfer groups, only the 8 and 13 hour transfers bloomed and set seed. Blooming began July 28 and rapid enlargement of pods began about August 9. The cessation of growth in length of the stems occurred at about the time the pods began to enlarge. On the other hand, the 16 hour transfers failed to form flowers and fruits, and it is seen that the elongation of their stems continued uninterrupted through-

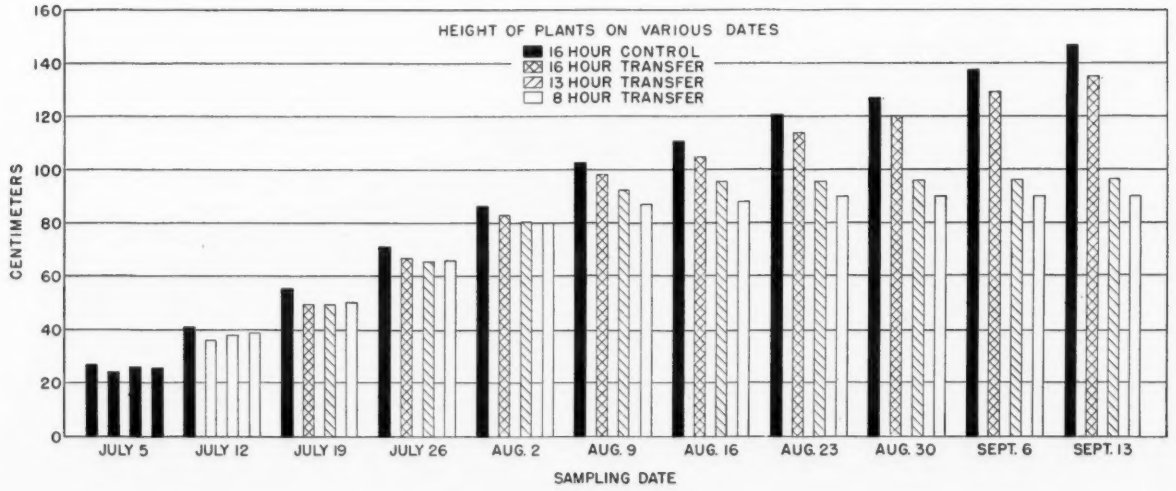

HEIGHT OF PLANTS ON VARIOUS DATES
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
CENTIMETERS
160
140
120
100
80
60
40
20
0
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
AUG. 16
AUG. 23
AUG. 30
SEPT. 6
SEPT. 13
SAMPLING DATE

FIG. 1

out the season and at a rate only slightly less rapid than that of the 16 hour controls.

Differences in the fresh weight of plants of the various groups began to appear as early as July 26 and became accentuated as the season advanced (table 6). On September 14, when the final sample was taken, the 8 hour transfer plants were nearing maturity and had begun to lose some of their leaves. Their total fresh weight at this time was somewhat less than it was on August 9. Fresh weight of the 13 hour transfers was approximately the same as on August 9.

No marked differences in total dry weight per plant occurred in the various lots until about August 2. From this time the 16 hour controls gradually accumulated more total dry weight than any of the other lots. The 16 and 13 hour transfers went through the season with approximately the same accumulation of dry weight per plant. On September 14 the 16 hour transfers were slightly in advance and both lots were definitely heavier than the 8 hour transfers.

On the first sampling date, July 5, the plants had all received 16 hour photoperiods for 6 weeks, and were therefore alike in so far as photoperiod treatment was concerned. Growing points of twenty-five plants were dissected and no flower primordia were present. There was an average of 20.4 nodes in the main axis of each plant.

On July 12 two samples were drawn, one from the 16 hour controls and one from the plants that had received 8 hour photoperiods for one week. No significant difference was found in total nodes in the two groups; the 16 hour controls had 23.1 nodes per plant and the 8 hour plants 23.2. The most striking difference between the two lots was that flower primordia were present on the 8 hour plants and absent on the 16 hour controls. Twenty-five plants were examined in each group and no exceptions were found.

The flower primordia were easily recognizable with a binocular microscope but were in a very early stage of development. The bract at the base of the pedicel of each flower and the two bracts that are attached to the sides of the flower just below the calyx were well developed. The calyx was just starting to differentiate, even in the most advanced primordia.

On July 19 there were still no flower primordia present on the 16 hour control plants. The 8, 13, and 16 hour transfers all had flower

primordia present July 12 that were induced by the 8 hour photoperiods of the week previous. During the week of July 12–19, the development made by these was influenced by the length of photoperiod to which they were subjected during that time. The buds on the 8 and 13 hour transfers were nearly three times the length of those on the 16 hour transfers. The mean lengths of twelve buds from comparable positions on plants of each of the three lots were 1.6, 1.7, and 0.6 mm. respectively for the 8, 13, and 16 hour transfers.

Aceto-carmine smears of the anthers of plants from the three groups were made. In the 8 and 13 hour transfers the microspore mother cells were full size and were separate from each other when pressed out of the anther. Early stages of meiotic division had not yet occurred. In the 16 hour transfers the anthers were very small and spore mother cells could not yet be recognized.

Although there were marked differences in the rate of growth of flower buds in certain of these lots during the week of July 12–19, there was a remarkable degree of uniformity in the number of new flower primordia initiated during that week. On July 12 flower buds were present in the buds of the main axis as high as node 19. A week later flower primordia were recognizable as high up the plant as node 23 in all three lots.

The average length of comparable flower buds on July 26 in the three transfer lots was 3.7, 3.9, and 0.7 mm., respectively. The 16 hour transfers had practically stopped development while the 8 and 13 hour lots had grown actively during the week. Pollen grains were present in the anthers of these two groups but no mother cells were yet visible in the 16 hour transfers. The controls were still without flower primordia.

On July 28 the first flowers opened on the 13 hour transfers. These were typical of flowers produced on soy beans under field conditions. The corollas of the 8 hour transfers on the other hand did not fully expand at anthesis. They pushed out beyond the calyx a short distance and remained in this condition for 2 or 3 days. The young pods then began elongation, indicating that pollination and fertilization had taken place. This behavior of soy beans grown on 8 hour days had been observed several times previously. Because of it the date of flowering cannot be determined in such plants. The

TABLE 6

PERCENTAGE MOISTURE AND DRY WEIGHT AND AVERAGE WEIGHT OF PLANTS RECEIVING VARIOUS PHOTOPERIODS

Photoperiodic treatment	Date sampled	Age of plant at time of sampling (days)	Percentage of moisture		Percentage of dry weight		Average weight per plant in grams							
							Fresh				Dry			
			Leaves	Stems	Leaves	Stems	Whole plant	Leaves	Stems	Pods	Whole plant	Leaves	Stems	Pods
Controls														
16 hour....	July 5	42	81.54	82.55	18.46	17.45	33.78	24.36	9.42	.....	6.13	4.49	1.64	.....
16 hour....	July 12	49	83.59	84.09	16.41	15.91	58.81	39.75	19.06	.....	9.55	6.52	3.03	.....
Transfers														
8 hour....			84.71	84.49	15.29	15.51	54.25	38.42	15.83	.....	8.32	5.87	2.45	.....
Controls														
16 hour....	July 19	56	84.14	83.25	15.86	16.74	93.68	63.96	29.72	.....	15.12	10.14	4.98	.....
Transfers														
16 hour....			83.60	83.31	16.40	16.69	84.31	58.10	26.21	.....	13.90	9.53	4.37	.....
13 hour....			81.63	81.83	18.37	18.17	72.45	50.57	21.88	.....	13.27	9.29	3.98	.....
8 hour....			84.58	83.58	15.42	16.42	83.95	58.48	25.47	.....	13.20	9.02	4.18	.....
Controls														
16 hour....	July 26	63	84.92	83.74	15.08	16.26	125.36	82.28	43.08	.....	19.40	12.40	7.00	.....
Transfers														
16 hour....			84.24	82.44	15.76	17.56	114.96	76.85	38.11	.....	18.80	12.11	6.69	.....
13 hour....			81.57	81.89	18.43	17.11	99.78	67.68	32.10	.....	17.96	12.47	5.49	
8 hour....			82.59	82.53	17.41	17.47	96.08	63.95	32.13	.....	16.74	11.13	5.61	.....

Controls														
16 hour....	Aug. 2	70	84.43	83.35	15.57	16.65	179.55	115.98	63.57	.....	28.63	18.05	1058.	.....
Transfers														
16 hour....			83.72	82.89	16.28	17.11	151.83	102.45	49.38	.....	24.72	16.28	8.44	.....
13 hour....			80.34	81.53	19.66	18.47	127.16	83.10	44.07	.....	24.46	16.33	8.13	.....
8 hour....			79.75	79.96	20.25	20.04	103.33	71.00	32.33	.....	20.84	14.37	6.47	.....
Controls														
16 hour....	Aug. 9	77	84.01	81.71	15.99	18.29	211.60	129.07	82.53	.....	35.73	20.64	15.09	.....
Transfers														
16 hour....			82.84	81.86	17.16	18.14	187.70	118.13	69.57	.....	32.89	20.27	12.62	.....
13 hour....			79.50	80.11	20.50	19.89	178.30*	103.87	57.55	16.88	34.25*	21.29	11.45	1.51
8 hour....			78.24	78.91	21.76	21.09	149.75*	80.65	39.21	29.89	27.51*	17.55	8.27	1.69
Controls														
16 hour....	Sept. 14	113	81.05	80.13	18.95	19.87	418.43	191.71	226.71	.....	81.38	36.33	45.05	.....
Transfers														
16 hour....			77.17	75.65	22.83	24.35	265.25	114.38	150.86	.....	62.84	26.11	36.73	.....
13 hour....			76.92	72.49	23.08	27.51	183.51*	46.20	55.41	81.90	53.41*	10.66	15.24	27.51
8 hour....			76.92	73.63	23.08	26.37	114.96*	34.06	22.48	58.41	39.02*	7.86	5.93	25.23

* Pods included.

fact that small pods made their appearance 3 or 4 days earlier than on the 13 hour transfers suggests that pollination and fertilization may have taken place slightly earlier in them than in the 13 hour transfers. The flower buds on the 16 hour transfers that were initiated during the induction period made no further development in most cases, but finally abscissed. In a few plants flower buds on some of the lower branches continued a slow development throughout the season and finally produced a few open flowers in September. During the latter part of July and during August no new flower primordia were formed on the 16 hour transfers. On September 14, however, many plants had again begun initiation of flower primordia in the axils of leaves near the terminals of the main and side branches. Initiation of flowers in similar positions on a few of the controls was also observed at this time. When further dissections of plants of both lots were made September 28, new flower primordia were found near the tips of all plants. These primordia were formed in spite of the fact that the plants had been kept continuously on 16 hour photoperiods except for one week in the case of the 16 hour transfers. The tendency of old Biloxi soy bean plants to initiate flowers even when kept on long photoperiods has been observed on several occasions.

The development of fruits and seeds on the 8 and 13 hour transfer plants proceeded normally, with the 8 hour plants somewhat in advance of the 13 hour ones throughout the remainder of the season. By August 9 the pods were 2–3 cm. long on both lots, and by September 14 the seeds were well formed but not yet mature.

CHEMICAL RESULTS.—The results of the chemical analyses have been calculated as percentage of both fresh and dry weight and as milligrams of a constituent per plant. The results as percentage of fresh weight are shown in tables 7 and 8, and the results as percentage of dry weight are shown in figures 2 to 17. Space does not permit the inclusion of the data calculated as milligrams of a constituent per plant.

NITROGEN.—The percentage of total nitrogen in the leaves and stems of a vegetative Biloxi soy bean decreased with the age of the plant. Most of this decrease occurred during the first 2 weeks of the experiment and was greater in the stems than in the leaves. When

plants were abruptly transferred to photoperiods of 8 hours, the percentage of total nitrogen in the leaves and stems increased and for several weeks remained higher than that of similar plants receiving a 16 hour photoperiod. Late in the season when pods were maturing on the 8 hour plants, the percentage of total nitrogen in both the leaves and the stems decreased to less than that of the controls. When other similar plants were transferred to 13 hour photoperiods after the week's induction, the total nitrogen on a dry weight basis immediately decreased in the leaves to a percentage similar to that of the control plants. This condition existed until the pods began to enlarge. At this time an increase in the percentage of total nitrogen occurred that was more pronounced in the leaves than in the stems. The stems of the plants receiving this photoperiod had approximately the same percentage of total nitrogen as the plants receiving an 8 hour photoperiod during the entire experiment. If the percentage of total nitrogen is considered from a fresh weight basis, however, these plants receiving a 13 hour photoperiod contained more nitrogen throughout the experiment than the controls, since they contained less moisture. On this basis the 13 hour transfer plants approached the 8 hour transfer plants in their percentage of nitrogen in both leaves and stems. The plants that were transferred to a 16 hour photoperiod, after the week of induction, had a higher percentage of total nitrogen in their leaves the first week following the transfer than the plants that had received a 16 hour photoperiod continuously. After that time the percentage of total nitrogen in the leaves of these closely approached that of the 16 hour control plants. The percentage of total nitrogen in the stems of the 16 hour transfer plants followed the same general trend as the leaves.

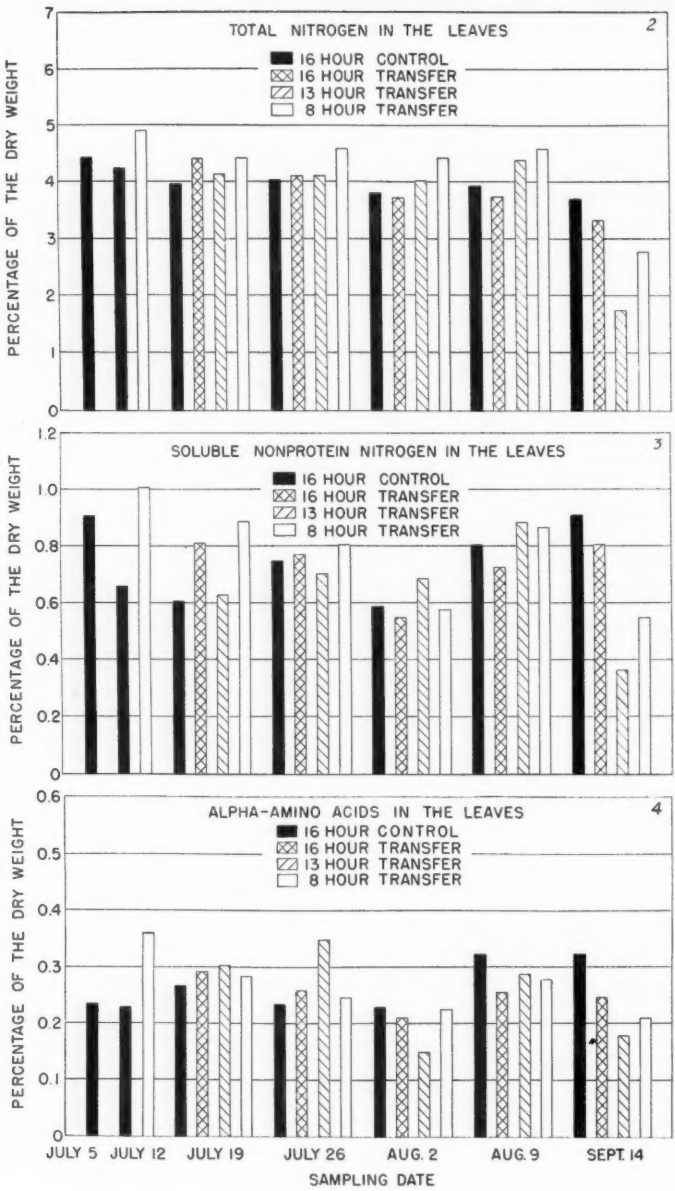
The actual milligrams of total nitrogen per plant increased as the plants became older. The differences between the milligrams of total nitrogen in the leaves of plants receiving a short photoperiod and those receiving a long photoperiod were slight during the induction period and until pods began to enlarge. As the pods matured the total nitrogen in the leaves and stems decreased. The stems of the plants receiving an 8 hour photoperiod contained less total nitrogen than those receiving a 16 hour photoperiod. This condition remained the same for the duration of the experiment.

TABLE 7

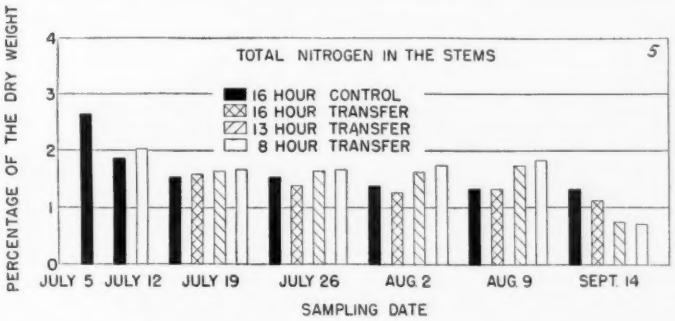
NITROGEN DISTRIBUTION IN LEAVES AND STEMS OF SOY BEANS SUBJECTED TO VARIOUS PHOTOPERIODS

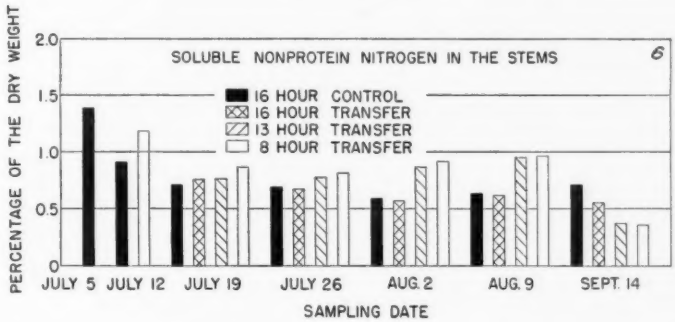
Photoperiodic treatment	Date sampled	Age of plant at time of sampling (days)	Percentage of nitrogen based on fresh weight of tissue													
			Total nitrogen		Protein nitrogen		Soluble non-protein nitrogen		Soluble organic nitrogen		Amino nitrogen		Ammonia nitrogen		Nitrate nitrogen	
			Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
Controls																
16 hour.....	July 5	42	0.826	0.458	0.659	0.215	0.167	0.243	0.108	0.156	0.043	0.084	0.003	0.005	0.056	0.082
Controls																
16 hour.....	July 12	49	0.702	0.300	0.594	0.155	0.108	0.145	0.085	0.114	0.037	0.050	0.005	0.006	0.018	0.025
Transfers																
8 hour.....			0.755	0.319	0.597	0.136	0.158	0.183	0.114	0.152	0.055	0.069	0.005	0.013	0.039	0.018
Controls																
16 hour.....	July 19	56	0.635	0.254	0.539	0.135	0.096	0.119	0.083	0.099	0.042	0.030	0.003	0.003	0.010	0.017
Transfers																
16 hour.....			0.728	0.310	0.595	0.161	0.133	0.149	0.107	0.120	0.047	0.051	0.003	0.002	0.023	0.027
13 hour.....			0.771	0.296	0.656	0.157	0.115	0.139	0.100	0.115	0.055	0.064	0.003	0.002	0.012	0.022
8 hour.....			0.690	0.275	0.553	0.133	0.137	0.143	0.114	0.118	0.044	0.053	0.005	0.004	0.018	0.021
Controls																
16 hour.....	July 26	63	0.615	0.246	0.502	0.133	0.113	0.113	0.099	0.098	0.035	0.049	0.002	0.001	0.012	0.014
Transfers																
16 hour.....			0.652	0.243	0.530	0.124	0.122	0.119	0.108	0.104	0.041	0.043	0.002	0.001	0.012	0.014
13 hour.....			0.763	0.279	0.633	0.147	0.130	0.132	0.120	0.119	0.064	0.042	0.002	0.001	0.008	0.012
8 hour.....			0.803	0.291	0.662	0.148	0.141	0.143	0.129	0.126	0.042	0.056	0.010	0.015	0.002	0.002

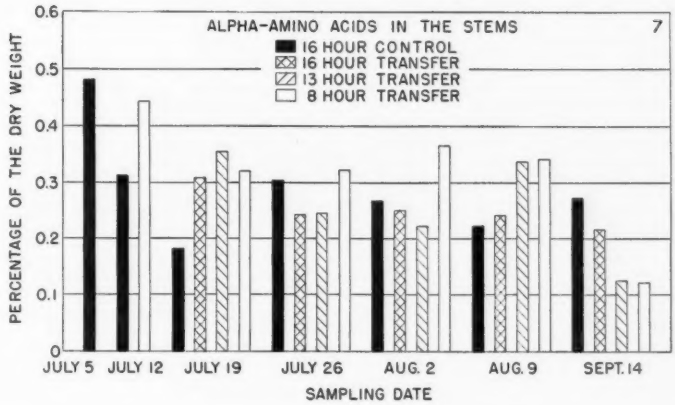
Controls																
16 hour.....	Aug. 2	70	0.599	0.223	0.507	0.124	0.092	0.099	0.079	0.083	0.035	0.045	0.002	0.002	0.011	0.014
Transfers																
16 hour.....			0.614	0.215	0.523	0.117	0.091	0.098	0.081	0.085	0.034	0.043	0.002	0.002	0.008	0.011
13 hour.....			0.801	0.297	0.666	0.137	0.135	0.160	0.122	0.131	0.029	0.041	0.011	0.025	0.002	0.004
8 hour.....			0.906	0.346	0.788	0.161	0.118	0.185	0.104	0.175	0.045	0.073	0.003	0.002	0.011	0.018
Controls																
16 hour.....	Aug. 9	77	0.633	0.241	0.503	0.124	0.130	0.117	0.110	0.097	0.051	0.041	0.002	0.002	0.018	0.018
Transfers																
16 hour.....			0.651	0.238	0.526	0.123	0.125	0.115	0.113	0.101	0.044	0.044	0.002	0.001	0.010	0.013
13 hour.....			0.908	0.347	0.726	0.156	0.182	0.191	0.170	0.177	0.059	0.067	0.003	0.002	0.009	0.012
8 hour.....			1.007	0.388	0.817	0.182	0.190	0.206	0.178	0.192	0.060	0.072	0.003	0.002	0.009	0.012
Controls																
16 hour.....	Sept. 14	113	0.711	0.261	0.537	0.121	0.174	0.140	0.168	0.134	0.061	0.054	0.003	0.002	0.003	0.004
Transfers																
16 hour.....			0.773	0.268	0.589	0.133	0.184	0.135	0.180	0.132	0.055	0.053	0.003	0.002	0.001	0.001
13 hour.....			0.413	0.203	0.326	0.100	0.087	0.103	0.082	0.099	0.041	0.035	0.003	0.002	0.002	0.002
8 hour.....			0.465	0.191	0.336	0.097	0.129	0.094	0.122	0.089	0.048	0.033	0.003	0.002	0.004	0.003


TOTAL NITROGEN IN THE LEAVES
2
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
1
2
3
4
5
6
7
SOLUBLE NONPROTEIN NITROGEN IN THE LEAVES
3
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
0.2
0.4
0.6
0.8
1.0
1.2
ALPHA-AMINO ACIDS IN THE LEAVES
4
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
0.1
0.2
0.3
0.4
0.5
0.6
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SAMPLING DATE

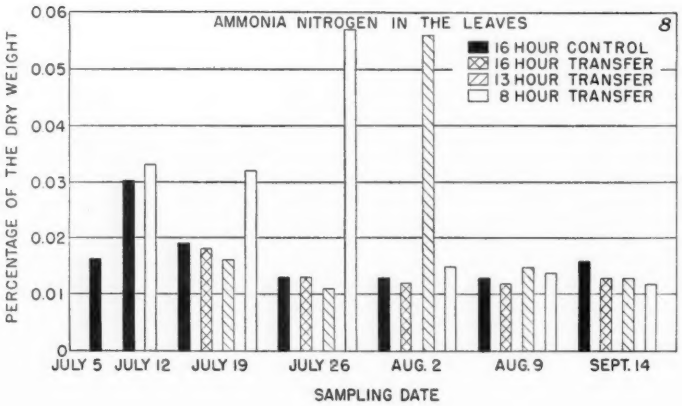
FIGS. 2–4

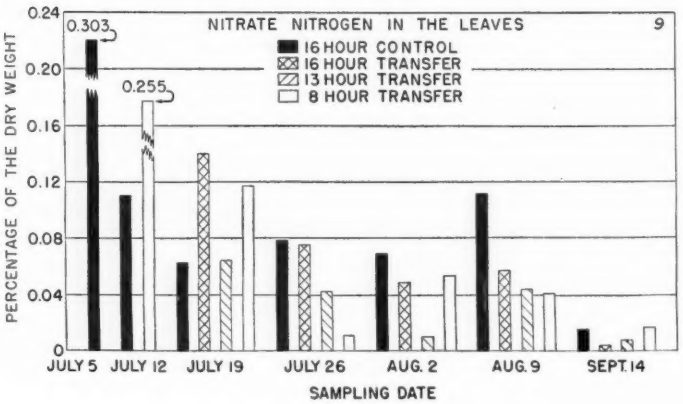

TOTAL NITROGEN IN THE STEMS
5
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
1
2
3
4
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SAMPLING DATE


SOLUBLE NONPROTEIN NITROGEN IN THE STEMS
6
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
0.5
1.0
1.5
2.0
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SAMPLING DATE

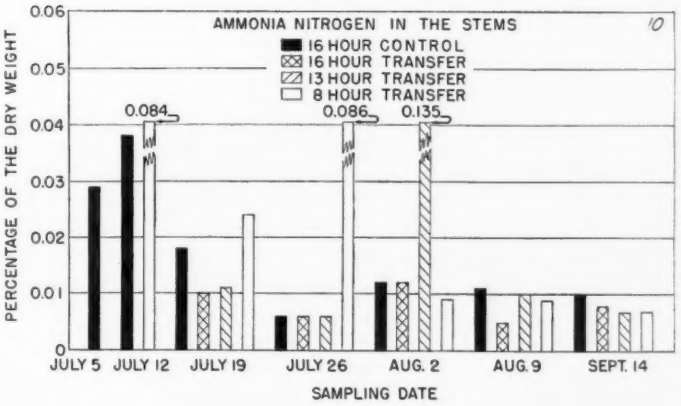

ALPHA-AMINO ACIDS IN THE STEMS
7
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
0.1
0.2
0.3
0.4
0.5
0.6
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SAMPLING DATE

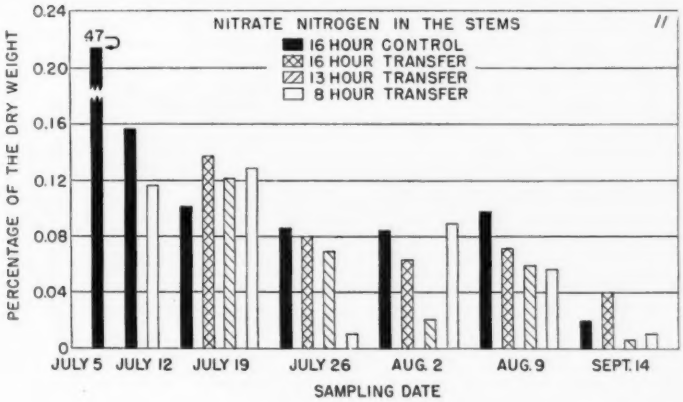
FIGS. 5–7


AMMONIA NITROGEN IN THE LEAVES
8
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0.06
0.05
0.04
0.03
0.02
0.01
0
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SAMPLING DATE

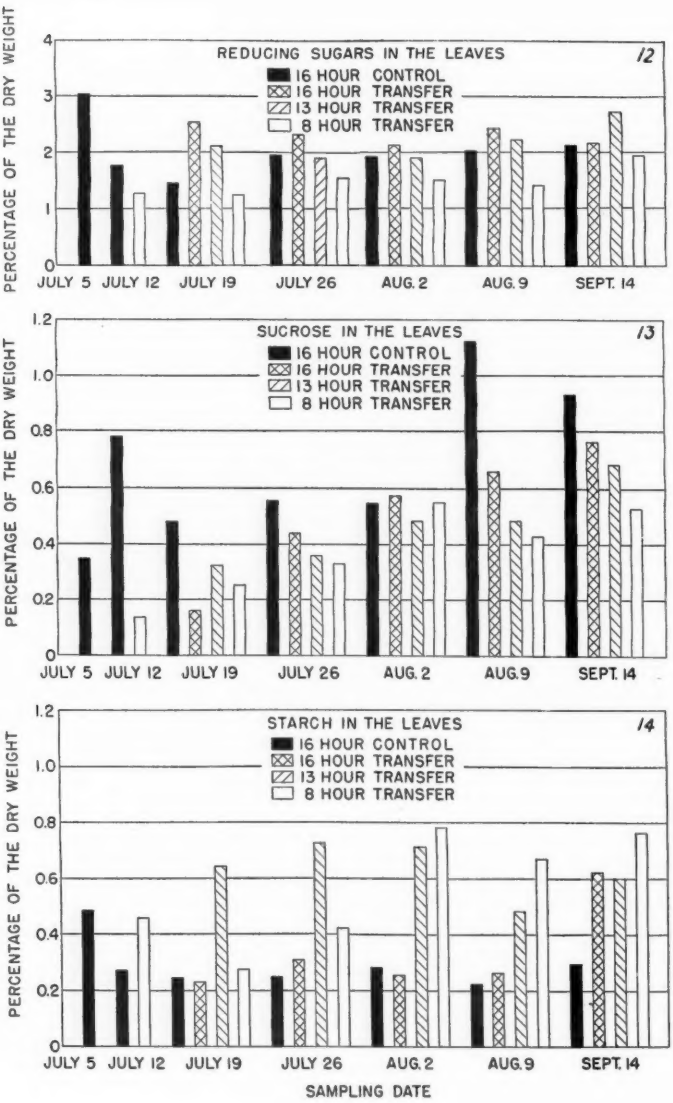

NITRATE NITROGEN IN THE LEAVES
9
0.303
0.255
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0.24
0.20
0.16
0.12
0.08
0.04
0
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SAMPLING DATE

FIGS. 8–9

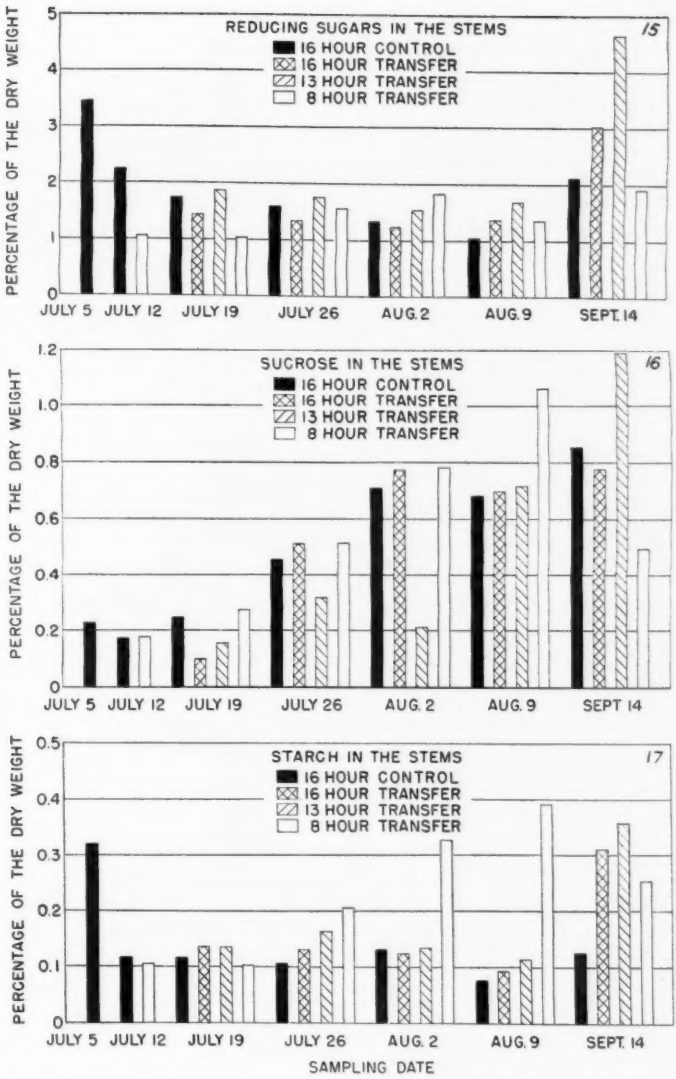

AMMONIA NITROGEN IN THE STEMS
10
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
0.084
0.086
0.135
PERCENTAGE OF THE DRY WEIGHT
0.06
0.05
0.04
0.03
0.02
0.01
0
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SAMPLING DATE


NITRATE NITROGEN IN THE STEMS
11
47
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0.24
0.20
0.16
0.12
0.08
0.04
0
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SAMPLING DATE

FIGS. 10–11


REDUCING SUGARS IN THE LEAVES
12
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
1
2
3
4
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SUCROSE IN THE LEAVES
13
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
0.2
0.4
0.6
0.8
1.0
1.2
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
STARCH IN THE LEAVES
14
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
0.2
0.4
0.6
0.8
1.0
1.2
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SAMPLING DATE

FIGS. 12–14


REDUCING SUGARS IN THE STEMS
15
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
1
2
3
4
5
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SUCROSE IN THE STEMS
16
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
0.2
0.4
0.6
0.8
1.0
1.2
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
STARCH IN THE STEMS
17
16 HOUR CONTROL
16 HOUR TRANSFER
13 HOUR TRANSFER
8 HOUR TRANSFER
PERCENTAGE OF THE DRY WEIGHT
0
0.1
0.2
0.3
0.4
0.5
JULY 5
JULY 12
JULY 19
JULY 26
AUG. 2
AUG. 9
SEPT. 14
SAMPLING DATE

FIGS. 15–17

During the induction period the percentage of the soluble non-protein nitrogen in the plants receiving an 8 hour photoperiod became greater than the controls in both leaves and stems. Those plants that remained on an 8 hour photoperiod following induction had a higher percentage of soluble non-protein nitrogen in the leaves on a fresh weight basis than the controls, until the time when pods were maturing. The percentage of soluble non-protein nitrogen in the leaves and stems of the 13 and 16 hour transfer plants was higher than that of the 16 hour controls and not greatly different from the 8 hour transfers, at the end of the first week following induction. Beginning with the second week after induction, the amounts of soluble non-protein nitrogen in the stems of 8 and 13 hour transfers approached each other and remained similar throughout the remainder of the experiment. The amounts of this constituent in the controls and 16 hour transfers showed a similar behavior. In the leaves on a dry weight basis greater fluctuations occurred, but at the time pods were beginning to enlarge there was more soluble non-protein nitrogen in the 8 and 13 hour transfer plants than in the plants of the other two lots. As the pods became mature the percentage of soluble non-protein nitrogen decreased rapidly in both the 8 and 13 hour transfer plants. The actual milligrams of soluble non-protein nitrogen in the leaves and stems was not greatly different in the various lots until the pods were nearing maturity on the 8 and 13 hour transfer plants. At this time the soluble non-protein nitrogen decreased greatly in both leaves and stems in these two lots. The alpha-amino acids in the leaves and stems of plants that received an 8 hour photoperiod were greater than those of the 16 hour control plants at the end of the induction period. After this there were fluctuations but these did not seem to have any general trend until pods were maturing on the 8 and 13 hour transfer lots. At this time there was a decrease of amino acids in both leaves and stems of these lots.

The percentage of ammonia nitrogen in the stems of the plants receiving 8 hour photoperiods was much higher than the controls after the induction period. In the leaves this condition occurred the following week. From that time until small pods began to appear the amount of ammonia in both the leaves and stems remained higher

TABLE 8

CARBOHYDRATE CONTENT OF LEAVES AND STEMS OF SOY BEANS SUBJECTED TO VARIOUS PHOTOPERIODS

Photoperiodic treatment	Date sampled	Age of plant at time of sampling (days)	Percentage of carbohydrates based on fresh weight of tissue							
			Reducing sugar		Total sugar		Sucrose		Starch	
			Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
Controls 16 hour..	July 5	42	0.558	0.603	0.652	0.674	0.064	0.040	0.089	0.056
Controls 16 hour..	July 12	49	0.295	0.355	0.449	0.406	0.128	0.028	0.044	0.018
Transfers 8 hour..			0.198	0.162	0.233	0.203	0.021	0.028	0.070	0.016
Controls 16 hour..	July 19	56	0.308	0.291	0.407	0.354	0.076	0.042	0.039	0.019
Transfers 16 hour..			0.418	0.286	0.464	0.319	0.026	0.017	0.037	0.016
13 hour..			0.392	0.339	0.476	0.383	0.050	0.027	0.118	0.024
8 hour..			0.195	0.168	0.248	0.226	0.039	0.045	0.042	0.016
Controls 16 hour..	July 26	63	0.296	0.261	0.403	0.355	0.084	0.074	0.038	0.017
Transfers 16 hour..			0.366	0.233	0.457	0.342	0.069	0.080	0.049	0.023
13 hour..			0.352	0.303	0.439	0.377	0.066	0.055	0.134	0.028
8 hour..			0.274	0.278	0.349	0.388	0.057	0.090	0.073	0.036
Controls 16 hour..	Aug. 2	70	0.302	0.222	0.411	0.362	0.085	0.118	0.044	0.021
Transfers 16 hour..			0.349	0.211	0.464	0.364	0.094	0.133	0.041	0.021
13 hour..			0.375	0.290	0.493	0.447	0.095	0.039	0.141	0.025
8 hour..			0.308	0.370	0.441	0.552	0.111	0.157	0.158	0.065
Controls 16 hour..	Aug. 9	77	0.324	0.187	0.532	0.331	0.179	0.124	0.036	0.014
Transfers 16 hour..			0.418	0.248	0.556	0.396	0.113	0.126	0.045	0.016
13 hour..			0.460	0.339	0.585	0.504	0.099	0.142	0.090	0.022
8 hour..			0.312	0.287	0.426	0.537	0.093	0.224	0.147	0.082
Controls 16 hour..	Sept. 14	113	0.408	0.421	0.618	0.618	0.177	0.169	0.055	0.025
Transfers 16 hour..			0.502	0.743	0.707	0.972	0.174	0.189	0.142	0.076
13 hour..			0.634	1.278	0.827	1.670	0.158	0.328	0.138	0.099
8 hour..			0.456	0.509	0.607	0.668	0.122	0.130	0.177	0.068

than in the controls. After pods had appeared, the percentage of ammonia in both leaves and stems was similar to that of the control plants. The plants transferred to 13 hour photoperiods contained approximately the same amount of ammonia in the leaves and stems as the controls, except at the time when very young pods were forming. At this time the percentage showed an abrupt rise over that of the controls. The ammonia nitrogen in the leaves and stems of the 16 hour transfer and the 16 hour control lots was similar throughout the experiment.

When the plants were subjected to a week of 8 hour photoperiod the nitrate nitrogen became greater in the leaves and less in the stem than control plants receiving a 16 hour photoperiod. When young pods were beginning to form on the 8 and 13 hour transfer lots, nitrate nitrogen was lower in the leaves and stems than in the controls. The times of occurrence of the low nitrate concentration corresponded to the time when ammonia was higher in these plants.

CARBOHYDRATES.—The results of the carbohydrate analyses are shown in table 8 and figures 12 to 17. At the end of the induction period the reducing sugars were lower in the leaves and stems of the plants receiving 8 hour photoperiods than in the controls. At the same time the amount of sucrose in the leaves was lower than that in the controls but remained the same in the stem. It was also evident from the data that the starch in the leaves during this period was higher in the short day than in the long day plants. In the stems the percentage of starch was very similar, regardless of photoperiod. Consequently the lower sucrose content of the leaves of plants on a short day may have been due to incomplete hydrolysis of available starch. During the week following the induction period, the leaves of the plants transferred to a 16 hour photoperiod contained more reducing sugars than those of any other treatment. The plants transferred to a 13 hour photoperiod had the next highest percentage of reducing sugars, and were followed closely by the 16 hour control plants. The least reducing sugar in any treatment was in the lots receiving the 8 hour photoperiod. The stems of the plants transferred to a 13 hour photoperiod contained the most reducing sugar after the first week of transfer, and the group of plants receiving the 8 hour photoperiods the least. At the same time more starch ac-

cumulated in the leaves of the plants receiving the 13 hour photoperiods than in any of the other treatments.

At the end of the second week after transferring the induced plants to different photoperiods, the percentage of reducing sugars was still high in the leaves of the 16 hour transfer group. The stems of the 13 hour photoperiod plants still contained the most sugars but the stems of the plants in all the treatments except the 8 hour photoperiod showed a decrease in the percentage of reducing sugars during the second week. The stems of the plants receiving an 8 hour photoperiod showed a marked increase in reducing sugars and sucrose and these plants were just beginning to form pods. This condition did not occur in the plants receiving a 13 hour photoperiod, however, and they were in practically the same morphological condition. Starch was still more abundant in the leaves of the 13 hour transfer plants than in any other group, while the stems of the plants on 8 hour photoperiods contained the most starch. Here again was a condition that occurred at the time of pod formation in the 8 hour photoperiod group but was not evident in the 13 hour photoperiod group.

At the termination of the third week after transferring the induced plants, small pods were present on the plants receiving the 8 and 13 hour photoperiods. These pods were too small to remove and were included with the stem sample. The pods on the plants receiving an 8 hour photoperiod were slightly more developed than those receiving the 13 hour photoperiods. This morphological condition was correlated with a high starch content in the leaves of both the 8 and 13 hour transfer groups. The stems of the plants receiving the 8 hour photoperiod also increased in starch.

The pods on the plants receiving 8 and 13 hour photoperiods were 1–3 cm. long the following week and were sampled separately. The leaves of the plants in these two groups contained much more starch than the leaves of the plants receiving 16 hour photoperiods. The stems of the plants receiving an 8 hour photoperiod were still much higher in starch than those receiving a 13 hour photoperiod, although both groups of plants were developing pods.

After 9 weeks in different photoperiods, the 8 and 13 hour groups were maturing seed, while the 16 hour transfer group had not yet flow-

ered. The results of the analyses of the leaves of the 8 and 13 hour groups showed that the milligrams of starch per plant had decreased during the production of seed. The stems of the plants receiving 8 hour photoperiods also decreased in starch but the stems of the 13 hour transfer plants increased in starch. The 16 hour transfer group contained much more starch in both leaves and stems than the 16 hour control plants. This was the first time that these two groups had shown any marked differences in starch content.

Discussion

In this experiment an attempt has been made to establish two reference lines in so far as the chemical behavior of fruiting and vegetative soy beans is concerned. The plants for one of these have been maintained in a vegetative condition throughout the season by keeping them on long photoperiods. Flower primordia have been initiated on the plants of the other lot by transferring them to a short photoperiod of 8 hours and maintaining them on it throughout the remainder of the season. The composition of the two lots has been determined at frequent intervals. The other lots upon which flower primordia had been initiated in response to a week of 8 hour days were subjected to photoperiods just above and just below the critical, respectively, during the remainder of the season. The chemical composition of these was also determined on the same dates as the other two lots. Such a study permits a comparison of the influence of long and short photoperiods upon the metabolism of plants that already have flower primordia initiated. The plants in the group on photoperiods slightly below the critical became definitely similar to the 8 hour plants in several respects, while the plants on photoperiods above the critical more closely resembled the controls. In other characteristics such tendencies of these two lots were not found.

Both lots kept on photoperiods below the critical produced flowers and fruits. The other two lots remained without flowers until the last of September. Even then flowering was restricted to the 16 hour transfers, and occurred on only occasional plants. No fruits were formed.

The two lots kept on photoperiods longer than the critical in-

creased in length throughout the season. The other two lots ceased to elongate at the time pods began to form.

The total fresh weight per plant became somewhat greater in the two lots grown at photoperiods longer than the critical than in the others. This difference was brought about chiefly by the greater moisture content of these two lots. In absolute amounts of dry matter the differences were not great. The two lots transferred to 13 and 16 photoperiods, however, maintained a position intermediate to the 8 hour transfers and the 16 hour controls.

As to the chemical composition, the data show that the total nitrogen in both leaves and stems of the 16 hour transfers became similar to the controls, and the 13 hour transfers approached the 8 hour ones as the season advanced. Protein nitrogen showed a similar relationship in both leaves and stems after pods were beginning to expand. The soluble organic nitrogen fraction showed similar difference in the stems but not so much in the leaves. The differences in this fraction in the stems were apparently due to compounds other than alpha-amino acids, since they did not show such a relationship until late in the season. The two lots of plants that fruited had a sharp but temporary increase in ammonia immediately after fruits began to form; the two non-fruiting lots did not have this sharp fluctuation. Simultaneously with the increase in ammonia the nitrates in the two fruiting lots were low.

With respect to their carbohydrate content, the two intermediate lots, which received the 13 and 16 hour photoperiod after induction, did not tend to become associated in composition with their respective reference groups as much as they did in respect to their nitrogen compounds. There was, however, a rather direct relationship among the three transfer groups in the reducing sugar content of the leaves and the length of the photoperiod. In the stems, on the other hand, the 13 hour transfers were higher than either of the other two transfer groups. The starch content of the leaves in the 8 hour and 13 hour groups and in the 16 hour transfers and control plants became similar after pods began to form, the former groups becoming much higher in starch than the latter groups. In considering these relationships it is necessary to bear in mind that the growth rates of the two lots receiving a 16 hour photoperiod were approximately

the same, and that they received the same number of hours of light and dark. The reserve carbohydrates were approximately the same in these two groups. The simple sugars were always higher in the leaves, however, and until later in the season lower in the stems of the plants that had received a week of short photoperiod. The reasons for this apparent difference in translocation cannot be explained from the data available. The growth of the 8 and 13 hour transfer plants was inhibited by their respective photoperiods, but these plants received different amounts of light for photosynthesis during the day and the following period of darkness was longer in the 8 hour than in the 13 hour group. Consequently the plants in the 13 hour transfer group always contained more reducing sugars in their leaves and stems than the 8 hour transfer plants. This relationship was also true of the reserve carbohydrates until pods began to enlarge; then the 8 hour plants had more starch than the 13 hour plants. This was probably due to the fact that the 13 hour plants set more fruit and as a consequence utilized more of the starch in their growth and development.

MURNEEK (**8**), working with Biloxi soy beans, reported that a high C/N ratio was not evident until the pods were maturing. He concluded that while a high C/N ratio was probably essential to normal fruit development, induction of flower primordia occurred when the ratio was lower than that of vegetative plants. In our work a higher percentage of nitrogen and a lower percentage of soluble carbohydrates occurred in the plants following the induction of flower primordia. There was an increase of starch in the leaves at this time. However, these differences in composition do not seem to have any direct causal relation to induction.

Following the induction period, the total carbohydrate and total nitrogen ratio in the leaves of the 16 and 13 hour transfers paralleled the 16 hour control plants while the ratio was lower in the 8 hour transfers. This ratio in the leaves of the plants bearing fruit was higher than the control only when the pods were maturing. The ratio of starch to total nitrogen was always higher in the leaves of the 13 hour transfers than in the controls, but the ratio in the 8

hour transfers did not become higher until pods were beginning to enlarge. The relationship of carbohydrates to nitrogen did not vary greatly until pods were either forming or maturing.

Summary

1. Biloxi soy bean plants with flower primordia initiated upon them were transferred to photoperiods of 8, 10, 12, 13, 14, 15, 16, and 18 hours. The development of these primordia and the flowering and fruiting responses of the plants were determined.

2. The plants transferred to photoperiods of 8 to 13 hours bloomed nearly simultaneously and all produced fruits. The yield of fruits on the 8 hour lot was somewhat less than on the 10, 12, and 13 hour lots.

3. Flowering on the 14 and 15 hour plants was later than on those of shorter photoperiod, the flowers were less numerous, and no fruits were formed.

4. No flowers opened on the 16 and 18 hour plants during the experiment.

5. When plants were transferred to a range of photoperiods after flower primordia were initiated it was found that the longest photoperiod on which fruit formation occurred was 13 hours and the shortest one on which no flowering took place was 16 hours.

6. Plants were grown for biochemical studies on 8, 13, and 16 hour photoperiods after initiation of flower primordia. A control lot was grown continuously on 16 hour photoperiods. The carbohydrate and nitrogen metabolism of these plants was determined at frequent intervals throughout the season.

7. At the end of the week's induction period the total nitrogen and soluble non-protein nitrogen were higher in the plants receiving 8 hour photoperiods than in the controls. Carbohydrates were lower than in the controls, with the exception of starch in the leaves which was higher.

8. The total nitrogen in both leaves and stems of the 16 hour transfers became similar to that of the controls and the 13 hour transfers approached the 8 hour ones as the season advanced. The soluble non-protein nitrogen showed the same relationship in the

stems. There was an abrupt rise in the amount of ammonia in the leaves and stems of the 8 and 13 hour transfer plants when pods were just beginning to form.

9. The amount of soluble carbohydrates in the transfer groups seems to be correlated with the length of photoperiod. Starch accumulated in the leaves and stems of the 8 and 13 hour transfers when pods were beginning to form.

10. Two groups of plants with flower buds initiated upon them and subsequently grown at photoperiods just above and just below the critical showed progressive deviation from each other in their carbohydrate and nitrogen metabolism. Those grown below the critical became similar to plants grown on 8 hour photoperiod, while those above the critical became similar to plants that had been kept vegetative by growing them continuously on 16 hour photoperiod.

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CURRENT LITERATURE

Submikroskopische Morphologie des Protoplasmas und seiner Derivate (*Submicroscopic Morphology of Protoplasm and Its Derivatives*). By A. FREY-WYSSLINGH. Protoplasma Monographien 15. Berlin: Borntraeger, 1938. Pp. 317. Figs. 138.

The author has set out upon the ambitious task of introducing the "average biologist" to the field of physics and biochemistry pertaining to cellular structure. Methods and terminology are given in the first hundred pages; the second hundred are devoted to the fine structure of cytoplasm, nucleus, and chloroplasts; the last hundred pages to the derivatives. Although the author is a plant physiologist, the third part of the book contains chapters on chitin, silc, keratin, tendons, muscle fibers and nerves. Cellulose, cutin, and starch grains receive equal attention.

The style of writing is fluent and often intriguing, and the many diagrams are without exception clear and illustrative.

The science of submicroscopic structures is concerned with the order of magnitude below 0.1 μ and above 1A, and it stands between cytology and the science of molecular structure. With new diagrams of old facts the reader learns or remembers about phases and phase-changes, molecular structure and molecular models. This prepares for a non-exhausting but to many probably instructive discussion of monolayers, liquid crystals, and myelin figures. There follows an important chapter on micellar science, which describes polymerization, especially of polysaccharides, chain-lengths, structural viscosity, contact points (Haftpunkte), and external micellar properties. Optical and x-ray methods are described, although the author's own field is primarily the former. "The micellar condition of a gel structure differs from the dispersoid in the fact that the constituent units can not be completely solvatised but remain attached at certain points of contact. With the rupture of these contact-points the micellar character is lost."

In the chapter on cytoplasm, polypeptide chains with side grouping showing various properties are described. Contact points derived from polypeptide chains may be of four kinds: (1) homopolar cohesions (mutual attraction of lipoid groups); (2) heteropolar cohesions (attractions of groups with dipole effect); (3) heteropolar valence bindings as salt or ester formation; and (4) homopolar valence bindings or bridge formation (S–S). Each type of binding is extensively discussed. pH and rH are discussed and compared as they affect micellar structure. There is a helpful discussion on fixation. "It is a fundamental difference between living and dead gels that in the former contact points are continuously under reconstruction." Perhaps more could have been said

on permeability, but the author offers a diagram; and following MEYER, a general formula is presented which takes into simultaneous consideration ion-mobility and lipoid solubility. Genes, chromosomes, and the spindle are discussed tentatively. A very searching treatment presents the author's most original fields, the chloroplasts and the cell walls built of cellulose, as well as the cutinized ones. The structure of starch grains is considered in the last chapter on reserve materials. In many cases the author has consciously over-simplified the problems, and few will have his identical approach to biology; but he has made it exceedingly easy for the reader to gain introduction at least, where previously only the specialist was admitted.—B. R. NEBEL.

The American Species of Crepis; Their Interrelationships and Distribution as Affected by Polyploidy and Apomixis. By E. B. BABCOCK and G. L. STEBBINS, JR. Carnegie Institution of Washington Publication No. 504, 1938. Pp. iv+199. Illustrated.

This is another of the recent striking examples of the integration of the findings of morphology, cytogenetics, ecology, and geographical distribution in a lucid and rational treatment of a difficult taxonomic complex, and in the formulation of plausible hypotheses on the origin and spread of the species and forms.

Seven adventive and twelve indigenous species of *Crepis* are recognized in western North America. The latter fall into three groups of 2, 1, and 9 species. *C. nana* and *C. elegans* in the first group are arctic-alpine forms of old world affinity ($x = 7$). The polymorphic but exclusively diploid *C. runcinata* ($x = 11$) forms the second, a wide-ranging species centering in the central Rocky Mts. The other nine species are best developed in the Columbia Plateau and northern Great Basin on arid, well drained hill and mountain slopes. These species are based on seven morphologically distinct and genetically isolated diploid forms ($2n = 22$) of somewhat restricted range, centering in northeastern California and adjacent Oregon, and central Washington, each occupying a particular habitat. These seven diploids are connected morphologically and geographically by a series of auto- and allopolyploid forms ($2n = 33, 44, 55, 77, 88$), generally apomictic. The presumed allopolyploids show their hybrid origin by combining the morphological characteristics and ecological tolerances of the presumed ancestry.

In the taxonomic treatment, the polyploid agamic complex caused the greatest difficulty. The authors chose an intermediate course between recognizing the entire complex as a single species and assigning specific rank to each apomict. Each diploid was taken to represent the morphological type of a species. "Those apomicts which fall (except for quantitative differences) within the range of variation of the diploids, as well as those partial allopolyploids which, by the possession of certain well-marked characteristics, show their undoubted affinity to one or other of the diploids, are assigned to the original species which they resemble. The other allopolyploid apomicts are grouped into agamospecies, each of which has approximately the same degree of variation

as the original species, and contains apomicts which appear to have originated from the same or similar hybridization."

"Within the species, the diploid form is carefully described and distinguished from the polyploids, and in addition the species is divided into subspecies or varieties on the same basis as are sexual species . . . . . The delimiting of species and subspecies has been done more or less independently of the nomenclatural history of the forms involved." Though the numerous apomicts have no taxonomic status, some of them have been named and described in the text under the species to which they were assigned. In addition, tables showing the characteristics in which they more closely resemble other diploids have been given for the named apomicts of each species.

The resulting treatment appears to be one which can be used satisfactorily by persons of differing botanical acumen, from those wishing only to assign a specific name to a plant in hand to those interested in the finest correlations of genetic constitution and environment.—C. E. OLMSTED.

Introduction to the Botany of Field Crops. Vol. I, *Cereals;* Vol. II, *Non-cereals.* By J. M. HECTOR. Johannesburg: Central News Agency, Ltd., 1936–1938. Pp. lxv+1127. Figs. 448. £3.10 per set.

In two volumes the author has assembled a wealth of material on a wide variety of economic plants, usually classed as field crops. The first volume is devoted to the cereals; first a chapter treating of them in general, then successive chapters dealing in detail with oats, wheats, rye, barleys, rice, millets, sugar canes, sorghums, and maize. Other grasses are considered very briefly if at all. The second volume deals with the Liliaceae, Moraceae, Polygonaceae, Chenopodiaceae, Cruciferae, Leguminosae, Linaceae, Malvaceae, Umbelliferae, Convolvulaceae, Solanaceae, Cucurbitaceae, and Compositae. Under each family there is a key to the several genera which contain species of economic importance, and at the end a comprehensive bibliography.

The treatment throughout is predominantly from a systematic and structural viewpoint, although a few details of ecological or physiological significance are added. In so far as material has been available for compilation, the entire plant has been considered—anatomically, histologically, and cytologically. The illustrations are many, mostly excellent but variable in this respect, partly owing to the quality of the original illustrations which have been copied and partly to the methods used in reproducing others. But the breadth of concept and outlook of this excellent work far outweigh any minor defects of illustration or typography. The author's thesis, as stated in the introduction, holds that a knowledge of form and structure of a plant enables the student better to understand its life processes; that the ecological viewpoint connects with the physiological. Familiarity with the reactions of a plant to its environment forms the groundwork for the study of crop ecology, a study which will pave the way to the development of an "adjusted agriculture." It is not too much to hope that such a goal may be attained, and these volumes constitute an effective aid toward such accomplishment.—K. C. HAMNER.

Native Woody Plants of the United States. By WILLIAM R. VAN DERSAL. Washington: Government Printing Office, 1938. Pp. 362. $1.75.

An indexed list of several hundred woody plants growing in the United States, naming each species as unmistakably as possible, and giving all available data pertinent to its use in erosion control and wildlife conservation. The United States is apportioned into thirty-two growth regions which are briefly characterized. The maps show respectively the plant growth regions of the United States, the climatic regions of Thornthwaite superimposed on Mulford's plant growth regions, and the important soil regions superimposed on the same growth regions. There are a number of good informative illustrations, an extensive bibliography, and a list of the common and scientific names of woody plants. —E. J. KRAUS.

Index to North American Ferns. By MAURICE BROUN. Orleans, Mass.: Published by the compiler, 1938. Pp. 217.

For more than three decades the study of North American ferns and their allies has increased the knowledge of habitats and ranges, created changes in nomenclature, and suggested rearrangements of genera and species to fit present day phylogenetic concepts. This Index is the first successful effort to assemble such information for use of pteridological amateurs as well as specialists. The book consists of a list of pteridophytes of North America (north of Mexico) arranged alphabetically by genera. Etymology and synonymy of names and the habitats and ranges of species are concisely given. The distribution of the species is so stated that the direction of its migration is evident. A systematic outline of the North American pteridophytes, a tabulation giving the number of introduced and native species (also forms and varieties), an author list, and an index complete enough to include synonyms add to the usefulness of the book.—P. D. VOTH.

La Distribution géographique des végétaux dans la région méditerranéenne française. By CH. FLAHAULT. Paris: Paul Lechevalier, 1937. Pp. xi+178. Maps.

Das Pflanzenleben der Ostalpen. By RUDOLPH SCHARFETTER. Vienna: Franz Deuticke, 1938. Pp. xvi+419. Figs. 73. Map.

A critical comparison of these two books indicates much of the development of geobotany in the last 40 years, including the elaboration of new concepts and the pulverization and refinement of old ones. The first volume has been printed from an unpublished manuscript of the late Professor FLAHAULT, which was awarded the *Prix Gay* in 1897. This manuscript and accompanying maps summarized in unified and simple fashion the author's own observations and researches of the preceding 16 years on the vegetation of the French Mediterranean region, corresponding in general to the range of *Quercus ilex* or its associated species in France. The editor, H. GAUSSEN, assures us that no other complete description of this region has ever appeared. In contrast, SCHARFET-

TER'S work is based upon the compilation of data from over 1000 references, and his observations during 30 years. His area is bounded by the Danube and the Po on the north and south respectively, a line running through the Lake of Constance and Lake Como on the west, and an irregular one from Pressburg through Agram and Laibach to Trieste on the east. The treatment is decidedly technical. The book is therefore of value only to persons familiar with the concepts, terminology, and some of the facts of central European geobotany, for whom it does serve as a not-too-well digested record and summary of the past researches on the region, and as a basis for future investigation. Valuable maps of the important communities of each region are found in the respective works.—C. E. OLMSTED.

An Introduction to Botany. By A. W. HAUPT. New York: McGraw-Hill Book Co., 1938. Pp. xii+396. Figs. 278.

HAUPT's definition of botany as "the science of plant life" indicates the scope of this book, the outstanding feature of which is its excellent correlation of structure and function. Since a knowledge of structure is necessary for an understanding of all other aspects of botany, morphology is emphasized; but stress is laid upon the concept of the plant as a living thing. The volume is intended to cover a semester's work; but it should serve equally well for a three months' course, especially a summer course, where so many of the students are teachers. The second part, with its evolutionary sequence, is particularly flexible and would be useful where a whole year can be devoted to the subject.

The illustrations are particularly good, most of them being prepared by the author himself.—C. J. CHAMBERLAIN.

Research and Statistical Methodology, Books and Reviews, 1933–1938. OSCAR KRISEN BUROS, Editor. New Brunswick: Rutgers University Press, 1938. Pp. vi+100. $1.25.

An assemblage of announcements, titles, and reviews of books on statistical methodology issued during the past five years. A work such as this is very useful, both because it presents the viewpoint and criticisms of several reviewers of any particular book, and because it brings these together in a single place where they are readily accessible instead of remaining widely scattered among numerous journals in different fields.—E. J. KRAUS.

Common British Grasses and Legumes. By J. O. THOMAS and L. J. DAVIES. London: Longmans, Green & Co., 1939. Pp. vii+124. $2.20.

While devoted more especially to the common grasses and legumes with which the British farmer is concerned, there is much in this book that is helpful to agronomists in many other countries, because of the wide distribution of many of the species listed. Workable keys to the vegetative characters of some common grasses and legumes, and clear cut and meaningful illustrations, make this brief volume readable and helpful.—K. C. HAMNER.